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# ON THE POSSIBLE ADAPTIVE SIGNIFICANCE OF MALE STERILITY IN PREDOMINANTLY INBREEDING POPULATIONS

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Received May 24, 1961

IN a theoretical study of the possible adaptive significance of gynodioecy as a breeding system, LEWIS (1941) had postulated that the cytoplasmic male sterility was favoured under natural selection due to an increased hybridization in the population. Later, DARLINGTON and MATHER (1949) argued that each species maintains a characteristic hybridity optimum in order to meet its opposing requirements of immediate fitness and long-term flexibility. This issue is particularly important in the case of predominantly self-fertilizing species where occasional bursts of outcrossing were assumed to originate the necessary hybridity (STEBBINS 1957). Recently, an analysis of changes in the genotypic frequencies at certain marker loci was carried out in a bulk-hybrid population of barley (JAIN and ALLARD 1960). It was found that a striking heterozygote advantage and one to two percent outcrossing together were able to retain substantial amounts of heterozygosity after 18 generations of selection and under as much as 98 to 99 percent selfing. In this paper it is suggested that naturally occurring male steriles in such a population might be adaptively favoured for their producing hybrid progeny and thus effectively contributing to an enhanced outcrossing. Such individuals, although reproductively less fit themselves, would therefore have a selective advantage due to their higher social fitness, or stated otherwise, they possess a higher average survival value in the population than the one expected on the basis of their individual effects (FISHER 1941).

In order to study the survival of male sterility factors with the help of a theoretical model, consider for simplicity the case of a recessive male sterile mutant *ms*, with genotypes *Ms Ms* and *Ms ms* fully male fertile. Let $P_n, Q_n, R_n$ denote the relative frequencies of *Ms Ms*, *ms ms* and *Ms ms* respectively in the $n$th generation, and assume the proportions of selfing ($s$) and random mating ($t$) to be .95 and .05, respectively. The selection coefficients for the genotypes *Ms Ms*, *Ms ms* and *ms ms* may be taken as $1-x$, 1, and $1-y$ which implies heterozygote advantage for $0 < x, y < 1$. Next, an inbreeding population in nature would not presumably hold too high a frequency of male steriles so that it seems natural to assume selection to favour male steriles below certain value of $q$ and disfavour above it. To put it in symbols, take either $x$ or $y$ to be a suitable function of $q$. One simple way of doing this is to equate $y$ to the value of $q$ in the preceding

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generation. Note that such a form of gene-frequency-dependent-selection would not allow fixation of *ms* or *Ms*. One other important assumption has to be made that there is increased probability of outcrossing on the male steriles, *ms ms*, say $kt$ $(k > 1)$, which would at least partly overcome the great disadvantage to the male steriles incapable of self-fertilization. Then, with $P_n$, $Q_n$, $R_n$ in the $n$th generation, the frequencies in the next generation $(P_{n+1}, Q_{n+1}, R_{n+1})$ are calculated as in Table 1. This results in the following set of proportionalities:

$$P_{n+1} \propto (1-x)\ \{s(P_n + \tfrac{1}{4}R_n) + t(P_n + \tfrac{1}{2}R_n)^2\}$$
$$Q_{n+1} \propto (1-q_n)\ \{s(\tfrac{1}{4}R_n) + t(\tfrac{1}{2}R_n)(kQ_n + \tfrac{1}{2}R_n)\}$$
$$R_{n+1} \propto s(\tfrac{1}{2}R_n) + t(P_n + \tfrac{1}{2}R_n)(kQ_n + R_n).$$

Given any set of initial genotypic frequencies $(P_o, Q_o, R_o)$, the recurrent use of these proportionalities would give the constitution of each of the following generations. In a population at equilibrium,

$$\frac{P_{n+1}}{P_n} = \frac{Q_{n+1}}{Q_n} = \frac{R_{n+1}}{R_n} = \text{a constant, } c;$$

this can give a set of three nonlinear equations in three unknowns (since $P + Q + R = 1$, $q = Q + \tfrac{1}{2}R$), namely $P, Q$ (or $R$) and constant $c$. The analytic solution of such a set of equations is apparently very difficult so that equilibrium values of $P, Q, R$ may be determined from the trend of actual numerical values in successive generations.

Several numerical examples of the foregoing model were worked out with different values of $x$ (viz. .1, .2, .3 and .4), $k$ (viz. 5, 10, 15 and 20) and of initial $P, Q, R$ (viz. .8, .1, .1; .90, .02, .08; and .98, .00, .02). In Figures 1–4 are plotted the values of $q$ in the first five generations for various combinations of $x$ and $k$. Three different initial values of $q$ were chosen to obtain a comparison among their rates of approach to equilibrium point which is uniquely determined by each pair of values of $x$ and $k$. However, it appears that in most instances, these gene frequency changes have to be computed for many more generations before equilibria could be attained. A few cases showing already a relatively slow rate of change in $q$ are given in Table 2. An important point to note is that the selective advantage of *Ms ms* over *Ms Ms*, as given by $x$, and the efficiency factor of outcrossing on *ms ms* individuals, $k$ seem to supplement each other in determining the value of $q$ at equilibrium. For instance, as indicated in the figures above, the combinations $(x = .2,\ k = 20)$ and $(x = .4,\ k = 5)$ seem to result in a rather low value of $q$ at equilibrium and percent heterozygosity

TABLE 1

*Derivation of genotypic proportions* ($P_{n+1}$, $Q_{n+1}$, $R_{n+1}$)

| Selfing part ($s$) | | | | | Random mating part ($t$) | | |
|---|---|---|---|---|---|---|---|
| | | Offspring | | | | Pollen | |
| Parent | Freq. | *Ms Ms* | *Ms ms* | *ms ms* | Ova | *Ms* | *ms* |
| *Ms Ms*: | $P_n$ | $sP_n$ | . . | . . | | $P_n + \tfrac{1}{2}R_n$ | $\tfrac{1}{2}R_n$ |
| *Ms ms*: | $R_n$ | $\tfrac{1}{4}sR_n$ | $\tfrac{1}{2}sR_n$ | $\tfrac{1}{4}sR_n$ | *Ms*: $P_n + \tfrac{1}{2}R_n$ | $t(P_n + \tfrac{1}{2}R_n)^2$ | $t(\tfrac{1}{2}R_n)(P_n + \tfrac{1}{2}R_n)$ |
| *ms ms*: | $Q_n$ | . . | . . | . . | $Q_n$ | $ktQ_n(P_n + \tfrac{1}{2}R_n)$ | $ktQ_n(\tfrac{1}{2}R_n)$ |
| | | | | | *ms*: $\tfrac{1}{2}R_n$ | $t(\tfrac{1}{2}R_n)(P_n + \tfrac{1}{2}R_n)$ | $t(\tfrac{1}{2}R_n)^2$ |

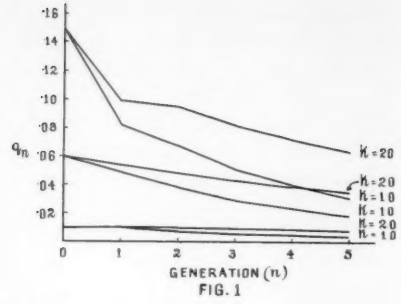

FIG. 1

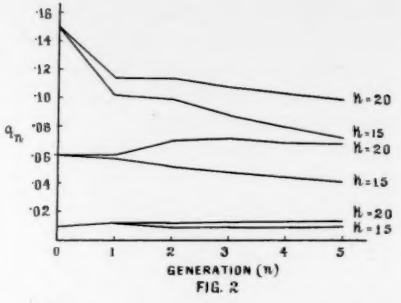

FIG. 2

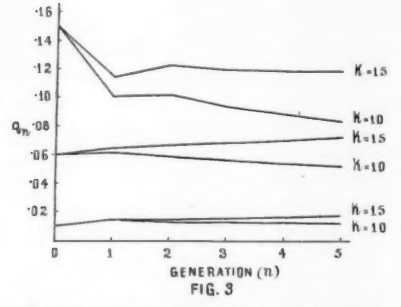

FIG. 3

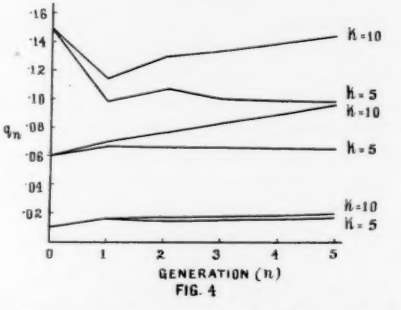

FIG. 4

FIGURES 1–4.—Curves showing changes in gene frequency ($q$) for $x = .1$ (Figure 1), $x = .2$ (Figure 2), $x = .3$ (Figure 3), and $x = .4$ (Figure 4).

TABLE 2

*Some numerical examples of the model (see text)*

| $x$, $k$ | Variable | Generation 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|
| | $R$ | .08 | .0744 | .0986 | .0868 | .0873 | .0844 |
| $x = .2$ | $q$ | .06 | .0602 | .0702 | .0713 | .0686 | .0672 |
| $k = 20$ | $R$ | .02 | .0131 | .0159 | .0153 | .0156 | .0157 |
| | $q$ | .01 | .0125 | .0119 | .0122 | .0123 | .0124 |
| | $R$ | .08 | .0718 | .0699 | .0669 | .0644 | .0619 |
| $x = .3$ | $q$ | .06 | .0616 | .0583 | .0561 | .0539 | .0517 |
| $k = 10$ | $R$ | .02 | .0149 | .0158 | .0153 | .0152 | .0150 |
| | $q$ | .01 | .0141 | .0129 | .0130 | .0127 | .0125 |
| | $R$ | .08 | .0755 | .0758 | .0754 | .0752 | .0750 |
| $x = .4$ | $q$ | .06 | .0672 | .0659 | .0658 | .0654 | .0652 |
| $k = 5$ | $R$ | .02 | .0173 | .0181 | .0184 | .0188 | .0193 |
| | $q$ | .01 | .0164 | .0151 | .0162 | .0166 | .0170 |

($R$) not any higher than that expected for $t = .05$ alone. On the other hand, ($x = .3$, $k = 15$) and ($x = .4$, $k = 10$) would give both $q$ and $R$ in a higher range of values that are indeed likely to be significant in the genetic structure of

predominantly inbreeding populations. Under such circumstances, male sterility becomes equivalent in effect to a higher rate of outcrossing in the population.

These theoretical results might be verified from an experimental study of the natural rates of occurrence of male sterility in inbreeders and the estimation of parameters $x$ and $k$. At least some of the above examples of $q$ and $k$ seem to be realistic in view of the work of RICK (1950) in tomato and of SUNESON (1951) in barley. Besides, it would be interesting to discover the type and amount of genotypic control of mutation and selection pressures for meeting hybridity requirements of natural populations.

## SUMMARY

In predominantly self-fertilized populations, certain male sterility factors may be adaptively important as a possible means of maintaining hybridity optima. A model is presented with heterozygotes selectively favoured and male sterile individuals having a higher probability of outpollinations than the average for the remainder population and with the help of several numerical examples it is shown that substantial amounts of heterozygosity might be retained in populations at equilibrium with suitable values of these parameters of selection.

## ACKNOWLEDGMENT

My sincere thanks are due to DRS. A. B. JOSHI and H. K. JAIN for their helpful comments.

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# BRISTLE FORMATION CONTROLLED BY THE ACHAETE LOCUS IN GENETIC MOSAICS OF DROSOPHILA MELANOGASTER[1]

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IN *D. melanogaster*, the genetic control of differentiation at specific sites is conveniently studied by the use of genetic mosaics. In wild-type flies, a definite number of macrochaetae or bristles is formed at specific sites of the thorax. Certain mutant genes are quite specific in their effects on bristle formation. The effect of achaete (*ac*), for example, is primarily restricted to removal of two bristles, the anterior and posterior dorsocentrals.

Earlier studies (STERN 1954a,b) have shown that bristle development is cell autonomous in mosaics. That is, in gynandromorphs in which the site of a posterior dorsocentral bristle is in *ac* tissue, a bristle will usually not differentiate even when most of the surrounding tissue is $ac^+$. Conversely, when the site of the bristle is $ac^+$, differentiation is always initiated regardless of the amount and distribution of surrounding *ac* tissue. This was interpreted by STERN as indicating that the hypodermal layer of the imaginal disc possesses regions in which the site of bristle formation is predetermined. A cell with the wild allele is competent to respond to this "prepattern," but a cell with the *ac* allele lacks this competence.

The present study of bristles affected by the *ac* locus uses a technique of mosaic production different from the one employed by STERN, one which produces a comparatively high proportion of mosaics thus permitting a quantitative breakdown of the various types of mosaics recovered.

## MATERIALS AND METHODS

Mosaic males were obtained from the cross $Dp(w^{vc})6094b/y\ w\ f$:=/Y females to $y\ ac\ w^a\ ct^6\ f \cdot Y^S/Y^L$ males. These males carried an X with the short arm of the Y attached and $Dp(w^{vc})6094b$ which is a small ring X duplication which arose from $In(1)X^{c2}$, $w^{vc}$ (HINTON 1955). The euchromatic composition of the duplication is limited to loci immediately adjacent to heterochromatin on each side of the centromere. It carries $y^+$, $ac^+$, and $w^+$. This duplication has a high rate of mitotic elimination during cleavage divisions, presumably through formation of dicentric double loop configurations at anaphase, thus forming yellow and white mosaic areas. Although there is no evidence of position-effect variegation of

[1] This research supported by U.S.P.H.S. Grant No. RG-7428.

yellow (HINTON 1955), there is white variegation as well as loss (BAKER, W. K., personal communication).

The X chromosome which carried achaete (*ac*, 0.0 +) was also marked by yellow (*y*, 0.0) so that achaete areas were recognizable by the presence of yellow bristles and hairs. As mentioned above, only wild alleles at the tip of the X were carried by the duplication so that *f* (forked bristles) could not serve as a marker of achaete tissue, but it is not difficult to recognize *ac* areas by the *y* marker alone. Since virtually 100 percent of the males from the above cross which did not carry the duplication had both anterior and posterior dorsocentral bristles missing, it was possible to score both bristle sites in mosaics.

## RESULTS

From the above cross, some 1600 males carrying the duplication were examined. Of these, 110 mosaics were found in which at least one of the four dorsocentral bristle sites or an area near a dorsocentral site consisted of *ac* tissue. The number of mosaics for the area considered represents approximately seven percent of the duplication carrying males.

Ninety-one of these mosaics exhibited autonomy; that is, when the bristle site is $ac^+$, a bristle always forms regardless of the amount of *ac* tissue surrounding the site. When the bristle site is *ac*, the result is less clear-cut. Two thirds of the autonomous cases have achaete areas uninfluenced by surrounding wild tissue. In most of these cases the wild tissue is not near the bristle site. However, 19 of the 110 cases were nonautonomous, in which dorsocentral bristles developed in *ac* tissue when $ac^+$ tissue was close to the bristle site. Since virtually all *y ac* males not carrying the duplication failed to differentiate bristles at the anterior and posterior dorsocentral sites, this cannot be due to lack of penetrance of the *ac* gene. STERN interprets this as being caused by spread of "$ac^+$ substance" into the *ac* tissue and considers it of minor significance compared with the larger number of cases of autonomy.

It should be noted that 12 of the 91 autonomous cases had similar closeness of $ac^+$ tissue to the bristle site with maintenance of autonomy, suggesting that distance over which substances must diffuse to the bristle site is not the only factor involved. Though nonautonomy is not infrequent, it is unrelated to the size of the *ac* area which may be a small patch at the bristle site or a large patch whose border passes through the bristle site. This again suggests, as brought out by STERN, that the alleles in question are not determining a pattern since area would be expected to be critical if such were the case.

Seven cases were observed in which a dorsocentral bristle differentiated in $ac^+$ tissue at an abnormal site. In each instance this was near a bristle site occupied by *ac* tissue. This phenomenon was interpreted by STERN as evidence that the property of the prepattern is distributed over a gradient field which has its peak at the typical bristle site but is still responsive at lower levels radiating out from this site.

The results obtained from mosaic males carrying Dp($w^{vc}$)6094b are consistent

with STERN's observations on gynandromorphs and his interpretation that *ac* and *ac*$^+$ are not establishing a regional singularity but are responding to a prepattern present in both genotypes.

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# THE CHROMOSOMAL LOCATION OF $Rf_1$, A RESTORER GENE FOR CYTOPLASMIC POLLEN STERILE MAIZE[1,2]

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Received April 17, 1961

COMPLETE restoration of pollen fertility to cytoplasmically pollen sterile maize in Texas (T) cytoplasm was shown by JONES (1951) to be mediated by at least one major dominant gene, and was later shown by DUVICK (1956) to require the simultaneous presence of at least two dominant genes, plus modifiers. The two major genes have been designated $Rf_1$ and $Rf_2$. $Rf_2$ is present in homozygous dominant form in most United States inbred lines of corn, whereas $Rf_1$ usually is homozygous recessive. BLICKENSTAFF, THOMPSON and HARVEY (1958) have reported that a major gene for full restoration of T steriles is located on chromosome 3 and that it has the following linear order and map distances with regard to anthocyanin-1 ($A_1$) and liguleless leaf-2 ($Lg_2$), two marker genes of chromosome 3:

$$A_1\text{—}36.2\text{—}Lg_2\text{—}20.8\text{—}Rf$$

In preliminary reports SNYDER (1955) and ANDERSON and DUVICK (1956) have also reported that a major gene for restoration of pollen fertility to T steriles is located on chromosome 3. SNYDER used recessive marker genes, and ANDERSON and DUVICK used endosperm-marked reciprocal chromosome translocations as described by ANDERSON (1952).

The present article amplifies the preliminary reports of SNYDER and of ANDERSON and DUVICK, presents additional data, and identifies the gene located by both methods as $Rf_1$. All genes referred to as $Rf_1$ and $rf_1$ in this presentation have been shown by means of appropriate testcrosses to be allelic to the recessive fertility restorer gene (designated $rf_1$) in the inbreds SK2, C106, and KA8. The restorer genetics of these inbreds has been described by DUVICK (1956).

## PART I. USE OF CHROMOSOMAL INTERCHANGES FOR LOCATING POLLEN RESTORATION GENES. MATERIALS AND METHODS

Several reciprocal translocation stocks from among those maintained at the California Institute of Technology were crossed to WG3, BH2 and KY21, three

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[2] This work was supported, in part, by a contract with the U. S. Atomic Energy Commission (At-04-3-41).

inbred lines which are homozygous for the dominant forms of $Rf_1$ and $Rf_2$. All translocations were closely linked to *wx*, on chromosome 9. The translocation stocks used were as follows:

| | | |
|---|---|---|
| 1-9a | 5-9a | 8-9a |
| 1-9c | 6-9e | 9-10b |
| 1-9 $_{4698}$ | 6-9c | |
| 2-9b | 7-9 $_{4363}$ | |
| 3-9c | 7-9a | |
| 3-9g | | |
| 4-9g | | |

The crosses (translocation × restorer) were then testcrossed as males to a T-sterile inbred of the waxy genotype *wx wx* $rf_1$ $rf_1$ $Rf_2$ $Rf_2$. The inbred contained all needed modifiers to $Rf_1$, so that all $Rf_1$—$Rf_2$—plants were fully fertile. All translocation stocks were found to be $rf_1$ $rf_1$, but were not tested for identity of the $rf_2$ gene. The tests with translocations were set up to identify linkages between translocations and $rf_1$.

The testcrosses were grown in both Iowa and California, or in Iowa only. They were classified as "fertile" or "sterile" and also as "normal" or "translocation heterozygote." The first pair of classifications was based on anther examination and refers to fertility restoration of cytoplasmic pollen sterility. The second pair of classifications was based on examination of open-pollinated ears and refers to the semisterility of eggs and pollen caused by a heterozygous translocation. Criteria for classification were as follows:

*Fertile:* Anthers normal, with 95–100 percent of pollen normal-appearing except in case of translocation heterozygotes, all of which (in the absence of cytoplasmic male sterility) have anthers with uniformly pebbly surface and about 50 percent of aborted pollen grains.

*Sterile:* Includes all completely pollen-sterile plants plus all grades of partial pollen fertility except that due to translocation heterozygotes as described above.

*Normal:* Full seed set on open-pollinated ears.

*Translocation heterozygote:* Scattered seed set, about 50 percent of possible kernels.

In most seasons, hot dry weather reduced the fertility of partial fertiles and it was relatively easy to divide plants into fertile and "sterile" classes. In one season, however, to be discussed further, partial fertility was sufficient to cause some problems in classification.

## RESULTS AND DISCUSSION

Both of the 3-9 translocations showed a close linkage with $rf_1$. No linkage was found between $rf_1$ and any of the other translocations. Table 1 summarizes the tests with the 3-9 translocations. Progenies within a particular testcross have been combined, for none differed significantly within a testcross pedigree. Chi-

TABLE 1

*Summary of testcrosses involving chromosome 3–9 reciprocal translocations and* $Rf_1$

| Pedigree | Location | Year | Classification: Parental NF* No. plants | Parental TS No. plants | Recombination NS No. plants | Recombination TF No. plants | Percent N | Percent F | Percent recombinations |
|---|---|---|---|---|---|---|---|---|---|
| Waxy $^T$(T3-9g × WG3) | Iowa | 1955 | 37 | 43 | 5 | 4 | 46 | 52 | 10 |
| Waxy $^T$(T3-9c × BH2) | Iowa | 1956 | 50 | 39 | 2 | 0 | 57 | 55 | 2 |
| | Calif. | 1956 | 30 | 26 | 0 | 1 | 53 | 54 | 2 |
| Waxy $^T$(T3-9c × WG3) | Iowa | 1956 | 259 | 277 | 7 | 9 | 48 | 49 | 3 |
| | Calif. | 1956 | 118 | 109 | 1 | 2 | 52 | 52 | 1 |
| Waxy $^T$(T3-9c × KY21) | Iowa | 1958 | 186 | 213 | 14 | 10 | 47 | 46 | 6 |

* NF—normal; fertile. TS—translocation heterozygote; sterile.
NS—normal; sterile. TF—translocation heterozygote; fertile.
See text for further explanation of classification types.

square tests showed that none of the values shown for percent normals and for percent fertiles differed significantly from the expected 50 percent.

The small population involving the 3-9g translocation had about ten percent of recombinants. The testcrosses involving translocation 3-9c had about one to three percent of recombinants except for that made with KY21 as source of restoration. Here, the percentage of recombinations was 5.7. This was significantly different ($x^2<.01$) from the pooled value for the other 3-9c tests. The other values did not differ significantly among themselves. The reason for the high recombination figure for the KY21 testcross is not clear. It differed from the others in several ways: (1) The initial cross to 3-9c was made at a different time, although going back to the same source; (2) the final testcross was made at a different time and place and was grown in a different season; (3) the number of partial fertiles in the testcross was higher than in the other testcrosses, and their fertility was so high that in some cases it was hard to know with certainty where to draw the line between full fertiles and "steriles." The standard used, that of calling only completely normal-appearing tassels "fertile" and all others "sterile" was arbitrary (in the absence of progeny tests of the classified plants), even though it did give an equal distribution of the two fertility classes.

Cytological studies by LONGLEY (1958) place the breakage points of the two 3-9 translocations as follows:

| | | |
|---|---|---|
| 3-9g | 3L.40 | 9L.14 |
| 3-9c | 3L.09 | 9L.12 |

All chromosome translocations tested involved chromosome 9 but since only those also involving chromosome 3 showed linkage with $rf_1$, it is apparent that $rf_1$ is on chromosome 3, rather than 9. The close linkage with 3-9c indicates that $rf_1$ is reasonably close to the centromere, probably on the long arm.

The close linkage with 3-9c also indicates that $rf_1$ probably is near tassel seed-4 ($ts_4$) at a maximum distance of about eight units since the 3-9c breakpoint has been shown to be closely linked (about 2.4 units) to $ts_4$ (E. G. ANDERSON, unpublished data). One must remember of course that suppression of crossing over

in the region of the translocation may have occurred, so that the genetic map distance could be greater than is indicated by this method.

## PART II. USE OF MARKER GENES FOR LOCATING POLLEN RESTORATION GENES.

### MATERIALS AND METHODS

The original cytoplasmic male sterile lines utilized in these tests were obtained from DR. D. F. JONES. The marker lines were obtained from the Maize Genetics Cooperative. The following recessive marker genes were used to test for linkage of restorer genes with chromosome 3: $ts_4$, $lg_2$, and dwarf-1 ($d_1$). The crosses made are indicated in Table 2, which shows the genotypes of the parents and of the $F_1$ progeny, as well as $F_1$ phenotypes and numbers of $F_1$ plants observed. The marker genes slashed (*sl*) and glossy seedling-1 ($gl_1$) were used to test for linkage of restorer genes with chromosome 7, and marker genes brachytic-1 ($br_1$), brown midrib-2 ($bm_2$) and anther ear-1 ($an_1$) were used to test for linkage with chromosome 1. Table 3 shows in detail the crosses made with these two sets of marker genes.

### RESULTS AND DISCUSSION

$Rf_1$ was shown to be linked with all of the marker genes on chromosome 3 (Table 4) with the closest linkage being to $ts_4$. Each of the allelic sets studied gave evidence of normal segregation. The $F_2$ ratios did not differ significantly from expected (Table 5). The linkage data reported earlier (SNYDER 1955), and shown in this report as Pedigree F, had indicated that $ts_4$ and $Rf_1$ were about one unit apart according to the recombinations between these two genes directly, but that they were about ten units apart by difference estimates involving linkage of each of these two genes with the other marker genes used. Linkage values derived from Pedigree F could have been inaccurate since (1) the cross was made in the repulsion phase, (2) the linkages of the marker genes are with a member of a segregating complementary pair of *Rf* genes, and (3) the $F_2$ population was quite small. In Pedigrees A through E, by way of contrast, one member of the complementary pair of *Rf* genes was homozygous dominant in both parents so that $Rf_1$ linkage values are based on 3:1 instead of 9:7 fertility segregations.

The more extensive data from Pedigrees A through E indicate that the estimate of ten units between $Rf_1$ and $ts_4$ was probably the most nearly correct. Differences between $Rf_1$-$ts_4$ recombination values for Pedigrees A through E (Table 4) were not statistically significant and it was determined by a $4 \times 5$ contingency table that the classification data are homogeneous. Therefore, the data were combined and a recombination value of about 10.5 percent was calculated from a total of 1570 $F_2$ plants. Similarly, the $Rf_1$-$lg_2$ and $Rf_1$-$d_1$ recombination values for Pedigrees B and D are not significantly different and it was found that these data are homogeneous. The combined data from a total of 510 $F_2$ plants gave recombination values of about 34.5 percent for the $Rf_1$-$lg_2$ linkage and 26.5 percent for the $Rf_1$-$d_1$ linkage. It is interesting to note that the sum of the recombination values for $Rf_1$-$ts_4$ (10.5%) and $ts_4$-$lg_2$ (23.6%) is approximately

TABLE 2

*Parental and $F_1$ data of cyto-sterile × chromosome 3 marker line crosses*

| Pedigrees | Parents entering crosses | Genotypes of parents | $F_1$ plants | $F_1$ genotypes | $F_1$ phenotypes |
|---|---|---|---|---|---|
| A | $C106^{T*} \times (Coop\ 49\text{–}26)^{TR}$† | $\frac{+\ rf_1}{+\ rf_1}\frac{+}{+} \times \frac{ts_4\ +}{ts_4\ +}\frac{+}{+}$ | 30 | $\frac{+\ rf_1}{ts_4\ +}\frac{+}{+}$ | all fertile |
| B | $(Coop\ 49\text{–}26)^{TR} \times (Coop\ 55\text{–}1220\text{–}7)\text{–}1$ | $\frac{ts_4\ +}{ts_4\ +}\frac{+}{+} \times \frac{+\ rf_1}{+\ rf_1}\frac{+}{+}$ | 30 | $\frac{+\ rf_1}{ts_4\ +}\frac{+}{+}$ | all fertile |
| C | $(Coop\ 49\text{–}26)^{TR} \times (Coop\ 55\text{–}1220\text{–}1)\text{–}1$ | $\frac{ts_4\ +}{ts_4\ +}\frac{+}{+} \times \frac{+\ rf_1}{+\ rf_1}\frac{+}{+}$ | 30 | $\frac{+\ rf_1}{ts_4\ +}\frac{+}{+}$ | all fertile |
| D | $C106^{T} \times (Coop\ 49\text{–}26)\text{–}3$ | $\frac{+\ rf_1}{+\ rf_1}\frac{+}{+} \times \frac{ts_4\ +}{ts_4\ +}\frac{+}{+}$ | 30 | $\frac{+\ rf_1}{ts_4\ +}\frac{+}{+}$ | all fertile |
| E | $C106^{T} \times (Coop\ 49\text{–}26)\text{–}2$ | $\frac{+\ rf_1}{+\ rf_1}\frac{+}{+} \times \frac{ts_4\ +}{ts_4\ +}\frac{+}{+}$ | 30 | $\frac{+\ rf_1}{ts_4\ +}\frac{+}{+}$ | all fertile |
| F | $C106^{T} \times (Coop\ 49\text{–}26)\text{–}1$ | $\frac{+\ rf_1}{+\ rf_1}\frac{+}{+} \times \frac{ts_4\ +}{ts_4\ +}\frac{rf_2}{rf_2}$ | 30 | $\frac{+\ rf_1}{ts_4\ +}\frac{rf_2}{+}$ | all fertile |

* "Texas" type cyto-sterile.
† Restored cyto-sterile tassel-seeded line.

TABLE 3

*Parental and $F_1$ data of cyto-sterile × chromosome 7 and chromosome 1 marker line crosses*

| Pedigrees | Parents entering crosses | Genotypes of parents | $F_1$ plants | $F_1$ genotypes | $F_1$ phenotypes |
|---|---|---|---|---|---|
| G | $Wf9^{T} \times (Coop\ 55\text{–}1220\text{–}7)\text{–}2$ | $\frac{+}{+}\frac{rf_1}{rf_1}\frac{rf_2}{rf_2} \times \frac{sl}{sl}\frac{+}{+}\frac{+}{+}$ | 30 | $\frac{+}{sl}\frac{+}{rf_1}\frac{+}{rf_2}$ | all fertile |
| H | $Wf9^{T} \times (Coop\ 55\text{–}1220\text{–}7)\text{–}3$ | $\frac{+\ +}{+\ +}\frac{rf_1}{rf_1}\frac{rf_2}{rf_2} \times \frac{gl_1}{gl_1}\frac{sl_1}{sl_1}\frac{+}{+}\frac{+}{+}$ | 30 | $\frac{+\ +}{gl_1\ sl}\frac{+}{rf_1}\frac{+}{rf_2}$ | all fertile |
| I | $C106^{T} \times (Coop\ 50\text{–}32\text{–}5)\text{–}1$ | $\frac{+\ +}{+\ +}\frac{rf_1}{rf_1}\frac{+}{+} \times \frac{br_1}{br_1}\frac{bm_2}{bm_2}\frac{+}{+}\frac{rf_2}{rf_2}$ | 30 | $\frac{+\ +}{br_1\ bm_2}\frac{+}{rf_1}\frac{+}{rf_2}$ | all fertile |
| J | $C106^{T} \times (Coop\ 51\text{–}80)\text{–}1$ | $\frac{+\ +}{+\ +}\frac{rf_1}{rf_1}\frac{+}{+} \times \frac{br_1}{br_1}\frac{an_1}{an_1}\frac{+}{+}\frac{rf_2}{rf_2}$ | 30 | $\frac{+\ +}{br_1\ an_1}\frac{+}{rf_1}\frac{+}{rf_2}$ | all fertile |

TABLE 4

*$F_2$ Crossing over values among chromosome 3 marker genes and between marker genes and* $Rf_1$

| Pedigrees | $ts_4$–$lg_2$ Percent | $ts_4$–$d_1$ Percent | $lg_2$–$d_1$ Percent | $Rf_1$–$ts_4$ Percent | $Rf_1$–$lg_2$ Percent | $Rf_1$–$d_1$ Percent |
|---|---|---|---|---|---|---|
| A | ..... | ..... | ..... | 8.0±2.8 | ..... | ..... |
| B | 19.5±1.7 | 23.0±2.1 | 35.0±2.5 | 15.5±4.1 | 33.0±3.6 | 25.5±3.8 |
| C | ..... | ..... | ..... | 9.0±3.3 | ..... | ..... |
| D | 26.5±2.3 | 33.5±2.6 | 49.5±3.2 | 12.0±4.2 | 36.5±3.7 | 27.0±3.9 |
| E | ..... | ..... | ..... | 0.0±6.5 | ..... | ..... |
| Combined pop. | 23.5±1.5 | 28.0±1.6 | 41.5±2.0 | 10.5±1.7 | 34.5±2.6 | 26.5±2.7 |
| F | 24.5±2.3 | 30.0±2.6 | 52.0±3.5 | 1.0±354.0 | 36.0±10.5 | 21.0±17.4 |

TABLE 5

*P values for 3:1 and 9:7 fertility and 3:1 chromosome 3 marker gene $F_2$ segregations*

| Pedigrees | Number plants | 3 fertile 1 sterile | 3 $Ts_4$ 1 $ts_4$ | 3 $Lg_2$ 1 $lg_2$ | 3 $D_1$ 1 $d_1$ |
|---|---|---|---|---|---|
| A | 553 | 0.10 | 0.95 | ... | ... |
| B | 260 | 0.10 | 0.80 | 0.50 | 0.60 |
| C | 400 | 0.70 | 0.30 | ... | ... |
| D | 250 | 0.80 | 0.95 | 0.85 | 0.15 |
| E | 107 | 0.80 | 0.02 | ... | ... |
| Combined pop. | 1570 | 0.02 | 0.75 | 0.60 | 0.60 |
| | | 9 Fertile 7 Sterile | | | |
| F | 213 | 0.20 | 0.98 | 0.40 | 0.08 |

equal to the $Rf_1$-$lg_2$ recombination value (34.5%). The $Rf_1$-$lg_2$ and $Rf_1$-$d_1$ values for population F are in reasonably close agreement with those for populations A through E, combined.

All recombination values were calculated by the product method (IMMER 1930). The $Rf_1$-$ts_4$ value was verified by the maximum likelihood method for the combination of data from several populations (ALLARD 1956).

No linkage was demonstrated between fertility restoration and marker genes on either chromosome 1 or 7. Chi-square tests of the recombination data indicated independent assortment between restoration and each of the marker genes employed. Thus, it is indicated that the second major restorer gene tested here (possibly $Rf_2$, although it has not been tested for allelism with the gene designated $rf_2$ in Wf9) is not located on the short arm of chromosome 7 nor on a considerable length of the long arm of this chromosome. It also does not appear to be located on a considerable length of the long arm of chromosome 1.

*Discussion concerning linkage data obtained by the use of marker lines:* Difficulty in obtaining backcross linkage data from crosses made in the coupling phase has arisen from the fact that pollen restoration is dependent upon two complementary *Rf* genes which can be heterozygous in both the cyto-sterile and marker lines. At the time these studies were conducted, two *Rf* genotypes were available,

namely, C106$^{T}$ (*rf*$_1$ *rf*$_1$ *Rf*$_2$ *Rf*$_2$) and Wf9$^{T}$ (*rf*$_1$ *rf*$_1$ *rf*$_2$ *rf*$_2$). The other homozygous genotype (*Rf*$_1$ *Rf*$_1$ *rf*$_2$ *rf*$_2$) needed for efficient testing of the *Rf* genotypes of marker plants is now available (DUVICK 1959). In addition, restored cyto-steriles of the *Rf*$_1$ *Rf*$_1$ *Rf*$_2$ *Rf*$_2$ genotype have been isolated. One such line is designated C106$^{TR}$.

In the future it is planned to make the Cyto-sterile × Marker line crosses in the coupling phase to facilitate the collection of backcross linkage data. To accomplish this and be certain which *Rf* gene is under test for linkage, the genotypes entering into the crosses will be as follows:

(1) For testing *Rf*$_1$—Marker linkage

C106$^{TR}$ (*Rf*$_1$ *Rf*$_1$ *Rf*$_2$ *Rf*$_2$ *MM*) × Marker (*rf*$_1$ *rf*$_1$ *Rf*$_2$ *Rf*$_2$ *mm*)

(2) For testing *Rf*$_2$—Marker linkage

C106$^{TR}$ (*Rf*$_1$ *Rf*$_1$ *Rf*$_2$ *Rf*$_2$ *MM*) × Marker (*Rf*$_1$ *Rf*$_1$ *rf*$_2$ *rf*$_2$ *mm*)

The *rf*$_1$ and *rf*$_2$ genotypes of the marker lines will be determined by testcrossing each marker line plant to cyto-sterile versions of (1) the *Rf*$_1$ *Rf*$_1$ *rf*$_2$ *rf*$_2$ and (2) the *rf*$_1$ *rf*$_1$ *Rf*$_2$ *Rf*$_2$ genotype. The F$_1$ progenies of these paired crosses will be all fertile and all sterile respectively, if the marker plant was *rf*$_1$ *rf*$_1$ *Rf*$_2$ *Rf*$_2$. They will be all sterile and all fertile respectively, if the marker plant was *Rf*$_1$ *Rf*$_1$ *rf*$_2$ *rf*$_2$.

## GENERAL DISCUSSION

The marker gene studies and the translocation studies have shown close agreement in placing the *rf*$_1$ locus in the proximal portion of the long arm of chromosome 3, not more than about 11 units from *ts*$_4$. The marker gene studies indicate further that the locus is to the left of *ts*$_4$, that is, between *ts*$_4$ and *d*$_1$. The *Rf* gene studied by BLICKENSTAFF *et al.* (1958) also was located in this general region, and it too may well be *Rf*$_1$, although published data do not list any crosses which definitely establish its allelism with the *rf*$_1$ locus as defined in this report. However, the linkage of *Rf*—*lg*$_2$ reported by BLICKENSTAFF *et al.* is 20.8 ± 2.3, whereas that of *Rf*$_1$—*lg*$_2$ reported here is 34.5 ± 2.6, which leaves an area of disagreement of about 14 units. It is possible that the vagaries of sampling, or unknown influences on recombination frequencies could be responsible for the different estimates or it is also conceivable that the *Rf* gene studied by BLICKENSTAFF *et al.* is at a different locus. An obvious next step is to cross one of the *Rf*$_1$ lines used in our studies with the one used by BLICKENSTAFF *et al.* and then testcross the F$_1$ to an *rf*$_1$ *rf*$_1$ tester such as C106$^{T}$. This is the procedure used to establish identity of the various *Rf*$_1$ genes studied by the two methods described in the present paper. An alternative would be to cross C106, SK2 or KA8 to one of the two cytoplasmic male sterile single crosses BLICKENSTAFF *et al.* used. If the three-way cross were male sterile, this would indicate that their *rf* source was indeed *rf*$_1$.

SUMMARY

*Rf*$_1$, one of two major dominant genes needed to restore pollen fertility to cytoplasmically pollen sterile maize (in Texas cytoplasm) has been shown to be on the proximal end of the long arm of chromosome 3, by means of tests involving chromosomal translocations and tests involving marker genes. Both methods place *Rf*$_1$ not more than about 11 crossover units from *ts*$_4$. The marker gene method gives the following map distances and gene order: *d*$_1$—27—*Rf*$_1$—11—*ts*$_4$—24—*lg*$_2$.

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# MODIFICATION OF ABNORMAL GAMETIC RATIOS IN DROSOPHILA. II. EVIDENCE FOR A MARKED SHIFT IN GAMETIC RATIOS IN EARLY VS. LATER SPERM BATCHES FROM A-TYPE BAR-STONE TRANSLOCATION MALES[1]

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FOLLOWING the finding of NOVITSKI and I. SANDLER (1957) that complementary gametic types from Drosophila males carrying the Bar translocation of Stone, T(1;4)$B^S$, are recovered with grossly unequal frequencies, it was reported by ZIMMERING (1960) that such inequalities were subject to considerable variation. It was shown that otherwise ordinary Y chromosomes and major autosomes derived from different common laboratory stocks were responsible for the variation, for in the presence of A ("abnormal ratios") Y autosome combinations marked inequalities are observed, while in the presence of E ("equal ratios") Y autosome combinations, the aberrant ratios tend to disappear. Males exhibiting the former kind of behavior were designated as the A type, and those exhibiting the latter kind as the E type.

The present paper concerns itself with evidence of an "age effect," in that while the first sperm released by young Bar-Stone males of the A type give clear evidence of aberrant ratios, those released later by the same males show more nearly equal ratios.

## MATERIALS AND METHODS

It may be well at this point to describe the translocation components of the $B^S$ male. Such a male possesses, as one pair of homologues, a fourth chromosome attached to a large distal piece of the X chromosome ($X^D$), and the normal minute-sized fourth chromosome (IV), and as another pair, the remaining proximal piece of the X chromosome ($X^P$) and the longer Y chromosome (Y). Four kinds of gametes are produced, namely, $X^D + X^P$, IV + Y, $X^D$ + Y, and IV + $X^P$. The gametic types $X^D + X^P$, IV + Y, and IV + $X^P$ are recovered from crosses with attached-X females usually carrying in addition a normal Y chromosome ($\overline{XX}$/Y), and the types IV + Y, $X^D$ + Y, and IV + $X^P$ are recovered from crosses with attached-X females carrying $X^P$ in place of the normal Y chromosome ($\overline{XX}$/$X^P$). From the values representing the relative frequencies of recovery of the four gametic types, the over-all frequency of each translocation component

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may then be extracted and comparisons made between the recovery of $X^D$ and IV, and between $X^P$ and Y.

All $B^S$ males employed in the present experiments were taken from a newly derived stock (June 1960) designated as an A type, since the males from this stock gave marked distortions in segregation ratios in each of two tests run previously. The results from each of these tests are given in tabular form and are compared with those from males taken from a stock designated as an E type.

| | $X^D$ | IV | $X^P$ | Y | n |
|---|---|---|---|---|---|
| Test 1—A type | 41.5 | 58.5 | 68.7 | 31.3 | 2086 |
| Test 2—A type | 40.5 | 59.5 | 67.0 | 33.0 | 2299 |
| E type | 52.6 | 47.4 | 49.1 | 50.9 | 3856 |

With reference to the results from the crosses involving males from the A-type stock, the following points are worth mentioning. The initial tests were made in a manner identical to previous tests; that is, by mating, on a small scale, a single male with a single tester female, and transferring the parents to fresh food at three-day intervals through the ninth day. Now, in these experiments, as in those conducted previously, while the *grand total* of offspring was sufficiently large to provide a fairly firm basis for calculating over-all frequency values, comparisons of frequency values *between* broods gave results of doubtful significance since the number of flies scored in each brood was small. On the other hand, a basis for comparison of gametic frequencies in different broods was provided from larger scale experiments to be described below.

*Results from the first experiment:* In the two experiments to be described, the males were a maximum of 24 hours in age when mated and the tester females were about 3–4 days old. The experiments were carried out at a temperature of about 25°C.

In the first experiment, single males from the A-type stock were mated each with three tester females. The parents were then simply transferred to fresh food every three days to make up three 3-day broods. The results from this experiment, as shown in Table 1, are only those obtained from males which produced offspring in all three broods. The data from the first three-day brood give convincing evidence of highly abnormal gametic ratios, apparent from the fact that IV is recovered about 50 percent more frequently than is $X^D$, and $X^P$ is recovered about 100 percent more frequently than is Y. However, the data from the second and third broods are clearly out of line with those from the first. It is evident that there was a sizeable change toward a more nearly equal recovery of the gametic types $X^D + Y$ and $IV + X^P$ from the first to the third brood, the difference in recovery rate between these two classes dropping from an initial 172 percent in the first brood to only 33 percent in the third brood. This change is reflected in the over-all recovery rate of the translocation components as evidenced by the decrease in the difference between $X^D$ and IV from 44 percent in the first brood to virtually no difference in the third, and by the decrease in the difference between $X^P$ and Y from 100 percent in the first brood to 34 percent in the third. Although changes in the relative frequencies of the gametic types

TABLE 1

*Progeny from three 3-day broods derived from matings of single males from the A-type* $B^S$ *stock, approximately 12–24 hours old, with three tester females*

| | $X^D + X^P$ | IV + Y | $X^D + Y$ | IV + $X^P$ |
|---|---|---|---|---|
| | 400 | 287 | (235) | 641 |
| | (450) | 323 | 245 | 668 |
| Brood 1 | 850 | 610 | 480 | 1309 |
| | 26.2 | 18.8 | 14.8 | 40.3 |
| | 1071 | 861 | (814) | 1293 |
| | (709) | 570 | 597 | 948 |
| Brood 2* | 1780 | 1431 | 1411 | 2241 |
| | 25.9 | 20.8 | 20.5 | 32.7 |
| | 780 | 578 | (659) | 880 |
| | (451) | 334 | 384 | 513 |
| Brood 3 | 1231 | 912 | 1043 | 1393 |
| | 26.9 | 19.9 | 22.8 | 30.4 |

Over-all frequency of recovery of each translocation component

| Brood | $X^D$ | IV | $X^P$ | Y | n |
|---|---|---|---|---|---|
| 1 | 41.0 | 59.1 | 66.5 | 33.6 | 3249 |
| 2 | 46.4 | 53.5 | 58.6 | 41.3 | 6863 |
| 3 | 49.7 | 50.3 | 57.3 | 42.7 | 4579 |

* Broods 2 and 3 were made up by simply transferring the parents successively from Brood 1. The data are only from males which produced progeny in all broods.

$X^D + X^P$ and IV + Y are not apparent in these results, this is not surprising since these two classes, even in the first brood, were recovered in frequencies so nearly equal that changes of a few percent tending to make them somewhat more equal would probably by chance alone not be demonstrable in this experiment.

*Results from the second experiment:* More precise information about this apparent gametic shift came from the results of the following experiment. Single males were supplied with three tester females every two days for a period of six days, thus making up three 2-day broods. After separation from the male, the females were transferred to fresh food and allowed to oviposit for an additional 3–4 days. The results from this experiment as shown in Table 2 were obtained only from males which gave offspring in all three broods. The following features of these results may be pointed out. (1) The gametic ratios are highly aberrant in the first brood, but (2) while the difference in the recovery rate between the gametic type $X^D + Y$ and IV + $X^P$ is about 190 percent in the first brood, it drops to only seven percent in the third. The somewhat more precipitous drop found in this experiment as compared with that found in the first is probably related to the breeding procedure, with that employed in this experiment permitting a greater precision in the delineation of successive sperm batches.

Briefly, then, each experiment has the following characteristics, namely, that while there is evidence of clearly aberrant gametic ratios in the first brood, there is a distinct trend toward more nearly equal ratios in subsequent broods.

TABLE 2

*Progeny from three 2-day broods derived from matings of single males from the A-type* $B^S$ *stock, approximately 12–24 hours old, with three tester females. Each brood was made up by crossing such males with three virgin tester females. The results are only from males which produced progeny in all broods*

| | $X^D + X^P$ | IV + Y | $X^D$ + Y | IV + $X^P$ |
|---|---|---|---|---|
| | 370 | 249 | (182) | 528 |
| | (190) | 134 | 110 | 320 |
| Brood 1 | 569 | 383 | 292 | 848 |
| | 27.2 | 18.3 | 14.0 | 40.5 |
| | 1277 | 1060 | (1218) | 1534 |
| | (779) | 647 | 707 | 890 |
| Brood 2 | 2056 | 1707 | 1925 | 2424 |
| | 25.3 | 21.0 | 23.7 | 29.9 |
| | 1358 | 1199 | (1318) | 1409 |
| | (717) | 633 | 710 | 759 |
| Brood 3 | 2075 | 1832 | 2028 | 2168 |
| | 25.6 | 22.6 | 25.0 | 26.8 |

Over-all frequency of recovery of each translocation component

| Brood | $X^D$ | IV | $X^P$ | Y | n |
|---|---|---|---|---|---|
| 1 | 41.2 | 58.8 | 67.7 | 32.3 | 2092 |
| 2 | 49.0 | 50.9 | 55.2 | 44.7 | 8112 |
| 3 | 50.6 | 49.4 | 52.4 | 47.6 | 8103 |

*The question of heterogeneity in the population of parental males employed:* The argument could be made that while on the whole the males employed in these experiments gave markedly abnormal gametic ratios in the first brood, some proportion of them were in fact of the E type. If the A-type males were, for example, less fertile in the long run and left considerably fewer offspring in the second and third broods than did the E-type males, this would result in a swamping of the A effect and give rise to what appeared to be a rather drastic shift in gametic ratios toward normality.

In partial answer to this argument, the over-all frequency of recovery of the translocation components from the first brood gives evidence of markedly abnormal ratios, and may be shown to be in at least fair agreement with those from previously published experiments which gave the *most* aberrant ratios thus far obtained from $B^s$ males. This is illustrated in a comparison with the data from NOVITSKI and SANDLER (1957).

| | $X^D$ | IV | $X^P$ | Y |
|---|---|---|---|---|
| Expt. I, Brood 1 | 41.0 | 59.1 | 66.5 | 33.6 |
| Expt. II, Brood 1 | 41.2 | 58.8 | 67.7 | 32.3 |
| NOVITSKI and SANDLER | 38.1 | 61.9 | 69.3 | 30.7 |

Thus, while there is an indication of somewhat more highly distorted ratios from the experiments of NOVITSKI and SANDLER, it would seem justified to suggest that

no large proportion of the males in the present experiments would be suspected of being of the E type.

As another approach to this question, the following analysis was carried out. Since the complementary gametic types $X^D + Y$ and $IV + X^P$ show the most marked shifts in their relative frequencies and thus serve as a sensitive measure of the extent of the shift from brood to brood, the ratio of these gametic types was calculated from the results of NOVITSKI and SANDLER (1957). The value of this ratio turns out to be approximately 27 percent. Consequently, on the basis of the data from the crosses of males with $XX/X^P$ females (from which these two types are recoverable) a selection was made of cultures where the value of this ratio in the first brood did not exceed 27 percent. Then, the value of the ratio was calculated for the second and third broods and compared with the corresponding ones derived from the offspring of all males used in these crosses. These results are shown from the selected cultures (s.c.) and from the population of males as a whole (p.w.).

| Expt. I | Brood 1 | Brood 2 | Brood 3 |
|---|---|---|---|
| s.c. | 44/268 = 16.4% | 220/398 = 55.3% | 151/223 = 67.7% |
| p.w. | 188/366 = 36.6% | 481/764 = 63.0% | 318/425 = 74.8% |
| Expt. II | | | |
| s.c. | 19/146 = 13.0% | 228/303 = 75.2% | 273/316 = 86.4% |
| p.w. | 110/320 = 34.4% | 709/892 = 79.5% | 712/761 = 93.6% |

Inspection of the table shows that the selected cultures in the first experiment gave a value of 16.4 percent in the first brood, as compared with 36.6 percent for the population as a whole, while the values from the second and third broods from the selected cultures were in rough agreement with those from the population as a whole—the comparisons being 55.3 percent (s.c.) *vs.* 63.0 percent (p.w.), and 67.7 percent (s.c.) *vs.* 74.8 percent (p.w.). The selected cultures in the second experiment gave a value of 13.0 percent in the first brood as compared with 34.4 percent for the population as a whole, and those from the second and third broods from the selected cultures were also in rough agreement with those from the population as a whole—the comparisons being 75.2 percent (s.c.) *vs.* 79.5 percent (p.w.) and 86.4 percent (s.c.) *vs.* 93.6 percent (p.w.). It may be suggested, therefore, that there does appear to be a real change in gametic ratios from early to later broods since the values in the later broods from the selected cultures were not only markedly higher than those in the first, but were higher than the value from the first brood based on the population as a whole, and were in at least rough agreement with the values derived from the population as a whole in the later broods.

As was indicated previously, it appears also that some males, initially used, may indeed have been of the E type since not only is there a consistently higher value in the second and third broods from the population as a whole as compared with those from the selected cultures, but the values in the first broods of 36.6 percent and 34.4 percent for the first and second experiments, respectively, are

higher than the value calculated from the data of NOVITSKI and SANDLER, namely, 27 percent.

*The probable major source of the E-type males:* Since the second experiment was conducted in a considerably more precise fashion than the first in that shorter brood periods were analyzed, and the males were supplied with fresh females for each brood, only the data from the second experiment were analyzed with the view of determining the major source of the E-type males. The following breakdown of the data provides a basis for a reasonably satisfactory answer to this question. Each of the 40 males employed in the crosses with $\overline{XX}/X^P$ females was characterized according to the total number of offspring representing the recovery of the gametic types $X^D + Y$ and $IV + X^P$ which were recovered in all three broods combined, and then simply arranged in ascending order according to this criterion. The males were then divided arbitrarily into two groups, one group representing that fraction of males which individually gave the fewer number of offspring, the other fraction the larger number of offspring. The kinds of divisions made were as follows: 1/2:1/2, 3/4:1/4, 4/5:1/5, 7/8:1/8, and 9/10:1/10. This breakdown is given in Table 3 with the associated values of the ratio $X^D + Y/IV + X^P$.

Simple inspection of the results in this table is sufficient to show that the ratio remained remarkably constant at a value of about 30 percent (not far out of line with the value of 27 percent discussed above), and was found ultimately to represent the average value for 36 of the 40 males used in this experiment. The remaining four males gave a high value of 71 percent in the first brood and probably represented the major source of the E-type males in this experiment. The ratio of the two gametic types for each of the three broods for the two fractions,

TABLE 3

*Groupings of males from the second experiment based on the total number of offspring in all three broods representing the recovery of the gametic types $X^D + Y$ and $IV + X^P$, with the associated values of the ratio $X^D + Y/IV + X^P$*

| Proportions* | n | (1) $X^D + Y$ | (2) $IV + X^P$ | (1)/(2) |
|---|---|---|---|---|
| F 1/2 | 1105 | 32 | 104 | .308 |
| L 1/2 | 2399 | 78 | 216 | .361 |
| F 2/3 | 1711 | 54 | 182 | .297 |
| L 1/3 | 1793 | 56 | 138 | .406 |
| F 3/4 | 2184 | 61 | 205 | .297 |
| L 1/4 | 1320 | 49 | 115 | .426 |
| F 4/5 | 2428 | 71 | 226 | .314 |
| L 1/5 | 1076 | 39 | 94 | .415 |
| F 7/8 | 2809 | 76 | 265 | .287 |
| L 1/8 | 695 | 34 | 55 | .620 |
| F 9/10 | 2944 | 90 | 292 | .308 |
| L 1/10 | 560 | 20 | 28 | .714 |

* F signifies that proportion of males yielding individually the fewer number of offspring, and L signifies that proportion giving the larger number of offspring.

i.e., 9/10 and 1/10, and those from the population as a whole are shown as follows.

| | | | |
|---|---|---|---|
| 9/10 | 90/292 = 30.8% | 573/762 = 75.2% | 565/654 = 86.4% |
| p.w. | 110/320 = 34.4% | 709/892 = 79.5% | 712/761 = 93.6% |
| 1/10 | 20/ 28 = 71.4% | 134/128 = 105% | 145/105 = 138% |

It is clear that a pronounced change occurred in the gametic ratios from the first to the third broods in the 9/10 fraction, and that the extent of the change is in excellent agreement with that found from the highly selected males (s.c.) referred to above which gave a value of only 13 percent in the first brood. The suggestion is therefore justified that the small number of highly selected males (10 of 40) and those representing 9/10 (36 of 40) of all males analyzed came from a population of genetically similar males.

## DISCUSSION

The following observations have been made: (1) sperm released by young $B^s$ males of the A type are characterized by marked distortions in the segregation ratios of the members of homologous pairs of chromosomes, in that IV is recovered about 50 percent more frequently than $X^D$, and $X^P$ is recovered about 100 percent more frequently than Y; while (2) sperm released 4–6 days later by the same males are characterized by a virtual absence of distorted ratios, in that $X^D$ is recovered almost as frequently as IV, and Y is recovered almost as frequently as $X^P$. In a formal sense, then, disjunction appears to be more normal in those groups of spermatocytes which are destined to form sperm used by the male about 5–7 days after hatching (or somewhat earlier), than in those groups of spermatocytes which form sperm used by the male about 1–3 days after hatching. This may be the result of some physiological difference(s) between these groups of cells, or between the groups of cells antecedent to them, such that in one instance, the expression of the A-type genetic factors is strong, and in the other, relatively weak or virtually absent.

Other examples of rapid changes with age may be noted here. These include such cases as (1) the relationship between the age of the male and the frequency of spontaneous mutations as reported by MULLER (1945) where newly hatched Drosophila males gave rise to a proportion of mutant offspring 2–3 times as high as did the same males tested a week or more later, and (2) the precipitous drop in the frequency of crossing over in the Drosophila female in approximately the first six days, from an initial high value of about 60–80 percent above the mean on the first day to a value of about 20–40 percent below the mean on the sixth day (SCHULTZ and REDFIELD 1951).

Finally, some speculation may be made about the possible bearing of these results on the finding by NOVITSKI and L. SANDLER (1956) that the change in the secondary sex ratio in humans with change in the age of the parents is more closely correlated with increasing age of the father than that of the mother. They conjectured that the simplest interpretation to account for this would be that the

relative frequencies of X- and Y-bearing sperm shift with the increase in age of the father so that the greater probability of younger fathers having sons is reversed in the case of older fathers. If the suggestion of a shift in gametic ratios as is indicated by the $B^s$ data has validity for human populations, this finding would lend support to that idea, since at least superficially, the shorter homologue in each case (the Y chromosome in man, and for example, $X^p$ in the $B^s$ case) tends to be recovered more frequently from younger males than are the longer homologues (the X chromosome in man, and Y in the $B^s$ case) while from older males both homologues tend to be recovered with more nearly equal frequencies.


## SUMMARY

Males of *Drosophila melanogaster* which carry the Bar translocation of Stone, T(1;4)$B^s$, possess as one pair of homologues a fourth chromosome attached to a large distal piece of the X chromosome ($X^D$) and the normal minute-sized fourth chromosome (IV), and as another pair, the remaining proximal piece of the X chromosome ($X^P$) and the longer Y chromosome. Evidence is presented which suggests that (1) sperm released by young $B^s$ males of the A type are characterized by marked distortions in the segregation ratios, in that IV is recovered about 50 percent more frequently than $X^D$, and $X^P$ is recovered about 100 percent more frequently than Y; while (2) sperm released 4–6 days later by the same male (provided the males are permitted to expend sperm in the meanwhile) are characterized by a virtual absence of distorted ratios in that all four translocation components are recovered with approximately equal frequencies. Conjecture is made that this may be the result of physiological differences between the groups of cells destined to give rise to the different sperm batches. Some speculation is made as to the possible bearing of the present results on the suggestion by NOVITSKI and SANDLER that that portion of the change in the secondary sex ratio in humans related to the change in parental age could be most simply interpreted as a shift in the relative frequencies of X- and Y-bearing sperm with a change in the age of the father.



## ACKNOWLEDGMENTS

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# A "MATERNAL EFFECT" ON BODY WEIGHT IN DROSOPHILA

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IN a study concerning the variability of response to selection for body weight in *Drosophila affinis, prima facie* evidence was found for the presence of a negative maternal effect (DEFRIES and TOUCHBERRY, unpublished). In this study, the regression of average weight of offspring on the weight of the male parent was found to be higher than that on the weight of the female parent. In addition, the presence of negative paternal half-sib components of variance and a "rebounding" phenomenon, i.e. large responses to selection followed small responses and *vice versa*, all could be explained by the action of a negative maternal effect.

Recently, MARTIN (1959) has reported a negative regression of fecundity of offspring on that of the parent in *Drosophila melanogaster* and suggested that this negative relationship may be due to the positive correlation of fecundity with body weight. It was hypothesized that females which oviposit more eggs inadvertently cause more competition among the resulting offspring and, hence, cause smaller body size. In turn, because of the positive correlation of body size and fecundity, these smaller female offspring are less fecund than average. However, the negative correlation found between body weight and egg size may also be involved in this relationship.

A somewhat similar situation regarding litter size in mice has been described by FALCONER (1955). An over-all regression of litter size of the daughter on that of the mother was found to be approximately zero. However, when the body weight of the daughter was held constant a regression of 0.10 was observed. Heritability, as estimated by doubling this latter regression, was found to agree quite closely with that realized from a selection experiment. That the daughter's weight is involved in this relationship was substantiated by the presence of a significant positive regression of litter size of the daughter on her own weight and a significant negative regression of daughter's weight on mother's litter size. Thus, there is apparently a positive genetic pathway and a negative nongenetic, i.e. maternal, pathway involved in the "inheritance" of litter size which tends to cause the overall regression of litter size of the daughter on that of the mother to be essentially zero.

MARTIN (1959) has also reported that the phenotypic variance of female *Drosophila melanogaster* weighed between zero and 24 hours after emergence was approximately twice that of females weighed between zero and 12 hours after emergence. However, the phenotypic variance of males weighed at the

different ages did not change appreciably. Thus, the change in the variance due to age in females was attributed to the considerable ovarian development which takes place between 12 and 24 hours after emergence.

The primary objective of the present study is to determine if there is a negative nongenetic pathway involved in the inheritance of body weight in Drosophila which operates through offspring number, i.e. number of adults to emerge.

## MATERIALS AND METHODS

The stock used in this study was derived from a large population of *Drosophila affinis* that was originally trapped in Urbana, Illinois, in 1959. The stock has since been maintained as a large random mating population in the laboratory, the mean daily temperature being maintained at approximately 76°F. All flies from which the data were taken were reared in 100 × 23 mm shell vials containing approximately one half inch of the usual corn meal-karo medium which was slanted and then inoculated with live yeast. The flies were weighed to the nearest microgram on a microbalance which had been found to give highly repeatable results. The intrasex repeatability, as determined by three repeated individual weighings of 25 males and 25 females, was found to be 0.96. As far as possible, all individuals were weighed between zero and 12 hours after emergence in order to reduce the variation caused by different stages of ovarian development in females. Because of differences in the time required for weighing, this interval occasionally was as large as 14 hours. Thus, the average age at which the flies were weighed was approximately six hours. Since the parameters to be estimated in the present study were to relate to a selection experiment, it was felt that the interval from emergence to time of weighing should be as short as possible. With 50 lines involved in this selection experiment, an interval of 12 hours was the shortest interval that was feasible.

## EXPERIMENTAL RESULTS

Samples of flies were drawn from the large random mating population by removing all flies from their containers, mixing them in an etherizing bottle, spreading them on a flat surface and then choosing individual flies on a basis of closeness to the experimenter until the desired number was obtained. The flies were then weighed and mated as single pairs, without regard to body size. This procedure was followed on each of five different days. From a total of 200 matings, however, only 183 produced at least five male and five female offspring. It was previously planned to use the average weight of the first five male and female offspring to represent offspring weight; therefore, data were collected from only these 183 matings. Offspring number was considered as the total number of adults to emerge within 16 days after the parents were removed from the vials; all parents were discarded when the first pupae appeared. In addition to the data collected from the 183 matings, there was also a considerable volume of data available from another study concerning the weights of parents and offspring; these additional data were included in the present study when relevant. Because

the data included in this study were collected during different periods, the data were analyzed on an intraperiod basis.

The relationships between weight of the male parent, weight of the female parent and number of offspring were investigated first. Therefore, only the data from the 183 matings were included in this portion of the analysis. The possibility of a correlation between the weights of the male and female parents was checked and an unexpected, but significant, correlation of 0.17 was found. Standard partial regressions of number of offspring on weight of the male parent, independent of weight of the female parent, and on weight of the female parent, independent of weight of the male parent, were then determined. These standard partial regressions were found to be 0.15 and 0.34, respectively, the former being significant at the 0.05 level of probability and the latter being significant at the 0.01 probability level. The statistical significance of the standard partial regression coefficients in this study was determined according to the method described by JOHNSON (1949).

The proposed relationship of weight of the male parent ($M$), weight of the female parent ($F$), number of offspring ($N$), and average weight of offspring ($O$) is illustrated as a path diagram in Figure 1. The paths (standard partial regression coefficients) from $M$ to $N$ ($b$) and from $F$ to $N$ ($c$) were determined as previously mentioned. The remaining paths were determined by equating the phenotypic correlations of $M$ and $O$, $N$ and $O$, and $F$ and $O$ to the respective paths contributing to these correlations. These correlations are shown in equations 1–3.

$$r_{MO} = a + be + rce + rd \quad (1)$$
$$r_{NO} = e + ba + cd + brd + cra \quad (2)$$
$$r_{FO} = d + ce + rbe + ra \quad (3)$$

Values for $r$ (the correlation between $M$ and $F$), $b$ and $c$ were substituted into the above equations. The phenotypic correlation between $N$ and $O$ was found to be $-0.081$ from data of the 183 matings. The additional data concerning 356 comparisons from the other study mentioned earlier were included in calculations regarding $r_{MO}$ and $r_{FO}$, however. These latter phenotypic correlations, therefore, are based upon 539 actual comparisons. However, the additional data are from a study covering three different periods, whereas the other data are from five

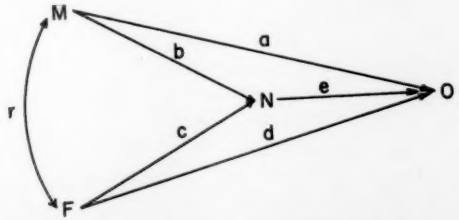


FIGURE 1.—Path diagram where $M$ is the body weight of the male parent, $F$ is the body weight of the female parent, $N$ is the number of offspring, $O$ is the average weight of the offspring and the smaller case letters represent the respective path coefficient.

different periods; therefore, since all analyses were done on an intraperiod basis, these correlations are based on 530 degrees of freedom. The correlations, $r_{MO}$ and $r_{FO}$, were found to be 0.085 and 0.002, respectively. Upon substitution of the respective values into equations 1–3, a set of three equations containing three unknowns resulted. This set of simultaneous equations was then solved, yielding estimates of the paths between $M$ and $O$ ($a$), $F$ and $O$ ($d$) and $N$ and $O$ ($e$). None of these paths was found to be significantly different from zero. Values for the paths and their standard errors are presented in Table 1.

## DISCUSSION

Several unexpected, but significant, relationships were revealed among the variables by the preceding analysis. The significant positive correlation between $F$ and $M$ indicates that some positive phenotypic assortative mating was involved in the pairing of mates and that these matings were not truly random. The highly significant path between $F$ and $N$ was expected on the basis of MARTIN's (1959) study, but the significant relationship between $M$ and $N$ was unexpected. This path ($b$) was determined independently of $F$; thus, it should not be attributed to the positive correlation between $F$ and $M$. If a causal relationship actually exists between $M$ and $N$ it would be interesting to learn its basis. It is possible that body weight of males is correlated to sperm production and that this could result in a positive relationship between body weight and offspring number.

The paths "$a$" and "$d$" may be regarded as the genetic pathways between the parents and offspring, whereas "$be$" and "$ce$" are nongenetic pathways. The heritability of body weight in Drosophila may be estimated by doubling the genetic paths, but such a method yields widely different estimates. Heritability, as estimated from the path between $M$ and $O$, is 0.20 and agrees with that reported by MARTIN (1959), whereas that determined from the path between $F$ and $O$ is only 0.04. Neither of the paths was significantly different from zero, and since both are positive in sign and have almost identical standard errors, it is not likely that they are significantly different from each other. However, if the estimates are close to the actual population parameters, it would be possible to rationalize a real basis for a difference. The phenotypic variance of female

TABLE 1

*Path coefficients*

| Path† | Value | Standard error |
|---|---|---|
| $M$ to $O$ ($a$) | 0.10 | 0.14 |
| $M$ to $N$ ($b$) | 0.15* | 0.07 |
| $F$ to $N$ ($c$) | 0.34** | 0.07 |
| $F$ to $O$ ($d$) | 0.02 | 0.15 |
| $N$ to $O$ ($e$) | −0.11 | 0.26 |

† $M$ is the body weight of the male parent, $F$ is the body weight of the female parent, $N$ is the number of offspring and $O$ is the average weight of the offspring.
* Significant at the 0.05 level of probability.
** Significant at the 0.01 level of probability.

Drosophila is approximately twice that of males (DEFRIES and TOUCHBERRY, unpublished) and much of this larger variance associated with females may be due to environmental differences. There is a considerable ovarian development in females with age, and differences in age at which the females were weighed could add to the environmental component of variance. Although all flies were weighed before 14 hours after emergence, and most within 12, it is possible that enough development occurred in some of the females before being weighed to increase the environmental component of variance and, consequently, reduce the heritability associated with the female parent. Of course, it is also possible that sex linkage or the lack of crossing over in males may also be involved. More data are necessary to determine whether the higher heritability associated with males is due only to sampling errors or if it is a real phenomenon.

The negative path between $N$ and $O$ was predicted, and although it is not significantly different from zero, it is believed to have a real basis. It is felt that competition and crowding can easily take place in the shell vials in which the flies were reared, even when single pair matings are used. Previous experience has shown that crowding certainly causes smaller body size, at least under more extreme conditions. However, it is not known how close the calculated path approximates the true population parameter.

Because of the highly significant path between $F$ and $N$ and because of the likelihood that a negative path exists between $N$ and $O$, it seems that the presence of a negative nongenetic pathway (maternal effect) in the inheritance of body weight has been verified. If the significant path between $M$ and $N$ has a real basis, it would appear that a negative pathway through the paternal side is also operating; however, the cause of a positive relationship between $M$ and $N$ is not as readily apparent as that between $F$ and $N$. As mentioned previously, it is possible that the body weight of males may be positively correlated to number of sperm produced and that this could result in a positive relationship between body weight of males and offspring number. Also, if body weight is positively correlated to sexual maturity, this could possibly account for the observed relationship. However, since all parents were left in the vials until pupae began to appear and since the maximum possible difference in age between flies chosen was 14 hours, it is not likely that this influence could be large. Nevertheless, the possibility of a nongenetic paternal effect is suggested.

As MARTIN (1959) suggested, it is possible that the negative nongenetic pathway may be caused by competition among the larvae. If competition or crowding is involved, the magnitude of this relationship should change when Drosophila are reared under different levels of environment. Thus, further studies in which containers of varying sizes are used or in which varying oviposition intervals are allowed should indicate whether such factors are involved in this interesting relationship.

## SUMMARY

From the results of this analysis, it may be concluded that a negative nongenetic path (maternal effect) exists in the inheritance of body weight in Dro-

sophila and that it operates through number of offspring. Path analyses revealed a highly significant positive relationship between body weight of the female parent and number of offspring and a negative relationship between number of offspring and body weight of the offspring. Empirical evidence had previously shown that this latter relationship, although not statistically significant in this study, is almost certainly real, at least under more extreme conditions. A small, but significant, positive relationship between body weight of the male parent and offspring number was also found, indicating the possible presence of a negative "paternal effect". Other relationships between body weight of male and female parent, number of offspring and body weight of offspring were presented. The possibility of a higher heritability regarding body weight in males than in females was also discussed.

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# VARIATIONS IN PREFERENTIAL SEGREGATION OF CHROMOSOME TWO IN TRIPLOID FEMALES OF DROSOPHILA MELANOGASTER

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PREFERENTIAL segregation in a triploid occurs when homologous chromosomes are not equivalent at the first meiotic division and the inclusion of certain homologous chromosomes into haploid and diploid gametes is not random. For example, if segregation is preferential, the homologous chromosomes $A_x$, $A_y$ and $A_z$ are distributed at meiosis so that the three segregations $A_x/A_yA_z$, $A_y/A_xA_z$ and $A_z/A_xA_y$ do not take place with equal frequencies.

STURTEVANT (1934, 1936) discovered preferential segregation in *Drosophila melanogaster*. He demonstrated the phenomenon with fourth chromosomes in triplo-4 females and in diplo-4 females with a chromosome fragment, Dp(1) scute-10-2. In his material the fourth chromosomes were from different sources but the preferential segregation could not be attributed to structural differences, because none was cytologically evident in salivary gland chromosomes.

Preferential segregation of second and third chromosomes in triploid females was reported by KOZHEVNIKOV (1940). Preferential segregation of the first chromosome was mentioned by SCHULTZ and REDFIELD (1951). In all of these cases, one chromosome of a triploid carried inversions that were inseparable from a dominant mutation. The other two homologues were structurally normal. The inversion-bearing chromosome was recovered preferentially in diploid gametes.

DOYLE (1959) described preferential segregation in trisomic, triploid and tetraploid inversion heterozygotes of maize. Structurally identical chromosomes were recovered in the same gametes less frequently than would have been expected had they segregated on a nonpreferential basis.

The data in this paper confirm KOZHEVNIKOV's and SCHULTZ and REDFIELD's observations that from triploid females an inversion-bearing chromosome tends to be preferentially recovered in diploid gametes when present with two normal homologues. A series of sequentially derived, rearranged second chromosomes were used in the present work to demonstrate that the more complex the rearrangement of the inversion-bearing chromosome, the greater the degree of preferential segregation.

## MATERIALS AND METHODS

*Chromosomes and stocks:* A stock with triploid females was obtained from the

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California Institute of Technology. The stock contained an attached-X chromosome marked with the mutants yellow-2, scute, apricot and echinus ($y^2$ *sc* $w^a$ *ec*) and the X chromosome balancer, FM4 (MISLOVE and LEWIS 1955). Triploid females of the stock possessed both chromosomes; males had only FM4.

All rearranged second chromosomes were marked with the dominant mutant, Curly. The following rearrangements were used:

1. Inversion (2L) with Curly *(In(2L)Cy)* is an inversion of the left arm of the second chromosome with break points at 22D and 33F-34A on the salivary gland chromosome map (BRIDGES and BREHME 1944).

2. Inversions (2L + 2R) found with Curly *(Ins(2L + 2R)Cy)* have inversions in both arms of the chromosome. One is *In(2L)Cy*, just described. The other is *In(2R)Cy* with break points at 42A and 58A on the salivary gland chromosome map (BRIDGES and BREHME 1944).

3. *SM1* was derived from irradiation of *Ins(2L + 2R)Cy*. Breaks at 22A and 60B superimpose a large pericentric inversion on *Ins(2L + 2R)Cy* (LEWIS and MISLOVE 1953).

4. *SM5* is the most complex rearrangement of this series of chromosomes. It is a product of irradiation on *SM1*. In addition to the break points listed for *SM1* there are seven more; at 21D1-2, 29CE, 36C, 40F, 42D±, 53C and 58F (MISLOVE and LEWIS 1955).

The positions of the breaks in the various rearranged chromosomes are shown in Figure 1.

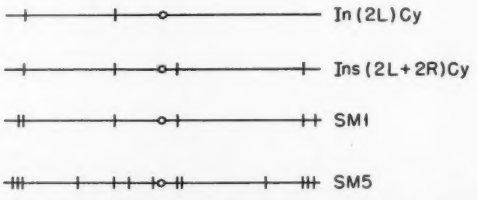


FIGURE 1.—Diagram of the break points of the series of rearranged second chromosomes used to determine degrees of preferential segregation. Symbols are defined in Materials and Methods.

*Experimental procedures:* Triploid females bearing $y^2$ *sc* $w^a$ *ec* and FM4 were crossed to males carrying one of the rearranged second chromosomes. Triploid daughters with the genotype $y^2$ *sc* $w^a$ *ec*/+; In/+/+ ("In" represents one of the rearranged chromosomes just described; "+" indicates a normal chromosome) were selected from among the progeny. Triploids with the rearrangement could be identified by their Curly phenotype, which is a reliable trait even when the mutant is present with two doses of its normal allele. These rearrangement-bearing triploid females were mated to (Canton-S) wild-type males.

The progeny of the inversion-bearing triploids were classified as having arisen either from eggs diploid for the second and third chromosomes (triploids and intersexes) or from eggs haploid for the second and third chromosomes (diploid

progeny). They were also classified as to whether or not they carried the rearranged second chromosome (*Cy* or non-*Cy*).

## RESULTS

The number of offspring of triploid females and the chromosome content of the egg nucleus from which they developed are listed in the first five columns of Table 1. In/+ and + gametes are reciprocal products of the same segregation,

TABLE 1

*Segregation from inversion-bearing triploid females*

| Chromosomes of female | Chromosome constitution of gametes: No. of diploid gametes In/+ | No. of diploid gametes +/+ | No. of haploid gametes In | No. of haploid gametes + | Frequency of segregation producing In/+ and + gametes | Frequency that In pairs |
|---|---|---|---|---|---|---|
| *In(2L)Cy/+/+* | 508 | 176 | 296 | 770 | 0.73 | .54 |
| *Ins(2L + 2R)Cy/+/+* | 422 | 131 | 307 | 1231 | 0.79 | .42 |
| *SM1/+/+* | 128 | 18 | 52 | 472 | 0.90 | .20 |
| *SM5/+/+* | 301 | 10 | 21 | 682 | 0.97 | .06 |
| If nonpreferential | ... | ... | ... | ... | 0.67 | .67 |

"In" represents a rearranged second chromosome; + represents a normal chromosome.

and the sum of their frequencies is a measure of the tendency of In to be recovered in diploid gametes and + to be recovered in haploid gametes. The frequencies of the In/+ ⟷ + segregations (In and a + chromosome to one pole of the first meiotic division spindle and a + to the other pole) are presented in the next-to-last column of Table 1. Under conditions of nonpreferential segregation the frequency of a In/+ ⟷ + segregation would be 0.67. *In(2L)Cy*, a simple inversion of the left arm of the second chromosome, causes a slight increase in the frequency of the In/+ ⟷ + segregation (0.73). The frequencies of the In/+ ⟷ + segregation increase as the complexity of the rearrangements increases. With the very complex *SM5* rearrangement the In/+ ⟷ + segregation occurs in 97 percent of the oöcytes.

The data may also be placed in terms of "effective pairing frequencies." The frequency that In pairs is listed in the last column of Table 1. On a nonpreferential basis, each of the three homologous chromosomes should be paired in 67 percent of the oöcytes. (Effective pairing is intended to indicate pairing that causes segregation of chromosomes to opposite poles of the spindle of the first meiotic division. A frequency of effective pairing is derived from analysis of genetic data and is not necessarily equivalent to cytologically visible chromosome associations.) The proportion of oöcytes in which the In is effectively paired is calculated by doubling the frequency of the In ⟷ +/+ segregation. This segregation is produced only after pairing of In with a +, but one half of the pairing between In and a + leads to a In/+ ⟷ + segregation (assuming that the unpaired + is equally likely to pass to either pole). The latter segregation is also the result of the pairing between two normal chromosomes.

*In(2L)Cy* reduces the pairing frequency to 0.54 and each increase in the complexity of rearrangement further decreases the pairing frequency. *SM5* is estimated to be paired in only six percent of the oöcytes.

## DISCUSSION

The preferential segregation in triploids observed in the data is interpreted as the result of a differential frequency of pairing, namely, the rearranged chromosome does not pair with a normal homologue as often as two normal homologues pair. The consequence is that normal chromosomes being paired, segregate from one another and are distributed to the two poles of the first meiotic division spindle. Therefore the rearranged homologue tends to be recovered in diploid gametes regardless to which pole it passes.

The way in which preferential segregation varies with chromosome structure in Drosophila may have some bearing on problems in plant genetics. The variation in segregation ratios obtained from synthetic amphiploids (summarized by GERSTEL and PHILLIPS 1958) has been attributed to the variation in differential affinity between homologous chromosomes derived from different species. Several authors (DARLINGTON 1937; STEBBINS 1950; GERSTEL and PHILLIPS 1958) have suggested that the basis of differential affinity lies in structural differences between chromosomes. Furthermore, STEBBINS (1956) suggested that fertility of autotetraploid plants could be increased—if preferential pairing could be increased—by induction of chromosomal rearrangements so that each chromosome is represented by two pairs of structurally different homologues. The data presented in this report substantiate these suggestions.

## SUMMARY

1. Triploid Drosophila females bearing one rearranged second and two normal second chromosomes produced progeny which indicated that the two normal chromosomes tended to pair and pass to opposite poles at the first meiotic division. The rearranged chromosomes were preferentially recovered in diploid gametes.

2. The extent of the preferential segregation was related to the extent to which the sequence of the rearranged second chromosome deviated from normal chromosome sequence. The more complex the rearrangement, the more frequently the rearrangement was recovered in diploid gametes. The greatest amount of preferential segregation was observed with the *SM5* chromosome. Only three percent of the diploid gametes did not contain *SM5*.

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# THE B BLOOD GROUP SYSTEM OF CHICKENS. II. THE EFFECTS OF GENOTYPE ON LIVABILITY AND EGG PRODUCTION IN SEVEN COMMERCIAL INBRED LINES[1]

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THE general existence of multiple allelism at the *B* locus and the retention of two or more *B* alleles following inbreeding (BRILES, ALLEN and MILLEN 1957; GILMOUR 1959, 1960) strongly suggest that some selection phenomenon favoring heterozygotes is operating in chicken populations. Working with the University of California production-bred flock of White Leghorns, SHULTZ and BRILES (1953) found that the *B* system heterozygotes were more frequent among females selected as breeders than in the original unselected population, indicating that artificial selection had, in general, favored the heterozygous types. Preliminary reports indicating that hatchability, livability, and egg production were affected by *B* blood group genotypes have been presented by BRILES (1954) and BRILES and KRUEGER (1955). The purpose of this paper is to present comparative data on survival and egg production of inbred chickens possessing different *B* system heterozygous and homozygous genotypes.

## MATERIALS AND METHODS

Seven inbred populations (or lines) were used in this study. The minimum coefficient of inbreeding for any individual within a line was .50 to .80, with the average level of inbreeding for each respective population being somewhat higher. These lines have been described previously by BRILES, ALLEN and MILLEN (1957) and will be referred to by the same designations as used in that publication—H1, H2, H3, H6, H8, H10, and H13. They constitute all of the lines used at one breeding station and can be considered to be representative of commercially useful inbreds. These lines have been used, or are at present being used, as basic parental stocks in the production of commercial inbred-hybrids. The seven lines originated from three breeds as follows: lines H1, H2, H8, and H10 are each from

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different strains of White Leghorns; line H3 is from a strain of New Hampshires; and lines H6 and H13 are from the same strain of Rhode Island Reds.

The serological techniques and the nomenclature used in identifying the B antigens present in these lines have been previously presented by BRILES, ALLEN and MILLEN (1957). It suffices here to state that reagents developed at the Texas laboratory for the A, B, C, D, and E systems (BRILES, BRILES and QUISENBERRY 1950; BRILES 1951, 1958) were used originally to detect red cell antigens in individual birds in each of the lines. On the basis of the blood type classifications resulting from these tests, B system reagents to be used for routine testing were prepared by isoimmunization within each of the respective populations. Agglutination tests performed on the red cells of individuals of each line with these intraline reagents disclosed that they effectively identified the *B* alleles previously designated on the basis of the Texas reagents.

The first generation of parents included in these studies were blood typed soon after they were placed in mating pens (housed in the fall of 1954), while the remaining two generations of parents were blood typed as chicks. All their progeny were blood typed between eight weeks and 150 days of age, usually at or near the latter age. Blood type information was not considered in selecting birds for mating nor in assigning them to breeding pens. Only families with four or more blood typed chicks surviving to 150 days are included in the summaries reported here. The number of *B* alleles originally disclosed continued to segregate in all subsequent generations without addition or loss of any alleles.

The data presented are from progeny performance records of matings made over a period of three years—1954, 1955, and 1956. Two shifts of males were used in reproducing the lines for each of the first two years, but only one shift was used in 1956. When two shifts were used, the females surviving the first were mated in the same pens with different males for the second. During each first shift 12 weekly hatches were produced from January 1 to March 15, and in each second shift 12 weekly hatches were produced from April 1 to June 15. There were 279 matings in the three years, resulting in 7732 fertile eggs and 3990 chicks that survived to 150 days posthatching.

Fertility was determined by candling individual eggs after seven days of incubation. Under this procedure it is usually expected that embryos dying from the time of fertilization to as late as the third or fourth day are not readily differentiated from infertile eggs; therefore, in this study it is probable that some eggs containing dead embryos were unavoidably classified as infertile.

No culling of either sex was exercised during the period from fertilization to 150 days of age. The progeny were moved from range to winter quarters when the age of the male shift of the seven inbred lines averaged 150 days. The pullets were housed ten to 15 birds per pen and mated with a single male. In each line there was an average of seven pens of pullets housed each year. Most of the males were housed separately from the females, with each line having one large pen of approximately 60 males. The birds to be housed were selected at random from those raised to housing age. The surplus birds were discarded and considered as

having never been present as adults; however, these surplus birds were considered to have completed the 150-day livability period. Any physically defective birds at housing were culled and considered as range mortality.

All birds dying during the three years were examined and, if possible, the cause of death was determined. If a bird died from an accidental cause on or before 150 days of age, the bird was eliminated as a housed pullet or cockerel. For the first year reported, culling was practiced throughout the laying period. Pullets were removed on the basis of unsatisfactory maturity, egg size, and rate of egg production. In summarizing the data, pullets culled during this first laying year were treated as accidental deaths. Considering the results to be presented, it is felt that any bias created by this culling in the first year would tend to obscure rather than magnify performance differences between blood type categories. No birds were culled in the second and third years. In each of the three years the recorded period of performance ended at approximately 450 days of age.

Egg production records were obtained by daily trap nesting. The period of recorded egg production began either October 1 or November 1 (depending on the year) and ended always on May 31 for both first and second shift pullets.

The results will be presented so as to facilitate the comparison of performance of blood group genotypes within lines; however, the data will also be pooled over the seven lines in an effort to estimate the possible over-all effect, as might be judged from a population consisting of distinctly different genetic isolates. To the extent that this composite population is representative of the species, the pooled data may be of value in indicating the effect, if any, of the *B* locus in non-inbred material.

The fertile eggs laid by any dam were apportioned into genotypes according to the genotypic ratio expected from the blood group alleles known to be present in the dam and sire. For example, if only the alleles $B^1$ and $B^2$ were involved in a particular mating and each parent possessed both alleles, the expected genotypic ratio for the zygotes would be 1 $B^1B^1$:2 $B^1B^2$:1 $B^2B^2$, and the fertile eggs from such a mating would be divided according to the ratio 1:2:1 into the three expected genotypic classes. In tabulating the expected zygotes from each type of mating, the quantity of eggs of each mating type was rounded to the nearest whole number. Only matings capable of producing two or more genotypic classes of progeny were included in the data to be presented on the survival of mixed sexes. Survival comparisons between genotypes within lines were all based upon the pooled expected numbers calculated for each of the full-sib classes. The genetic relationship resulting from these within-family comparisons, superimposed on the inbreeding already present within each of the populations, should make for considerable over-all genetic homogeneity between comparison groups. Also, comparing full-sib progeny has the advantage of minimizing maternal effects.

In analyzing and interpreting the viability results of this study, it was assumed that Mendelian segregation and fertilization occur at random with regard to the *B* locus. This is justified in general on the failure of non randomness of fertility

to have been reported in chickens and is justified especially for the *B* locus by the 1:1 segregation (1177:1197) in chicks from matings designed especially for linkage studies (BRILES, unpublished).

## RESULTS

*Juvenile livability:* The survival of zygotes from fertilization to 150 days of age (posthatching) will be referred to as juvenile livability; in contrast, survival from 150 to 450 days of age will be referred to as adult livability. Juvenile livability comparisons for the various *B* system genotypes in each of the inbred lines are shown in Table 1. The data for each comparison are limited to two-allele matings in which at least one parent was heterozygous, so that all matings summarized would be expected to produce a class of heterozygotes and at least one class of homozygotes. In comparing the livability of a heterozygote *vs.* a single type of homozygote, data from the two types of matings (one or both parents heterozygous) were combined. For example, in line H1, a total of 778 fertile eggs were laid in the three years by $B^1B^2$ females mated to $B^1B^2$ males. Apportioning these eggs on an individual family basis according to the zygotic ratio 1:2:1 resulted in an expected number of fertile eggs for each class as follows: 195 $B^1B^1$, 388 $B^1B^2$, and 195 $B^2B^2$. In this line no progeny were obtained from $B^1B^1 \times B^1B^2$ matings; however, $B^2B^2 \times B^1B^2$ matings produced 84 fertile eggs. Apportioning these according to the expected 1:1 ratio resulted in 42 fertile eggs for each of the $B^1B^2$ and $B^2B^2$ genotypic classes. Adding the chicks from this mating to those from the $B^1B^2 \times B^1B^2$ mating above resulted in a total expected number of fertile eggs of each genotype for the three comparison groups as follows: 195 $B^1B^1$ vs. 388 $B^1B^2$ (Table 1, part A), 237 $B^2B^2$ vs. 430 $B^1B^2$ (Table 1, part B), and 195 $B^1B^1$ vs. 195 $B^2B^2$ (Table 1, part C).

The procedure of comparing the heterozygote separately with each homozygote seems reasonable on the grounds that in a population a mutant (or migrant) allele is initially present only in the heterozygous state and an increase in frequency of the new allele would primarily depend on the selective advantage of the new heterozygous type over its respective homozygous counterpart originally present. As the new allele increases in frequency, it will eventually be exposed in the homozygous state, thereby pitting itself as a homozygote against the other genotypes present in the population.

To facilitate separate comparison of the homozygotes with heterozygotes over the seven inbred lines, some system of assigning the two homozygotes within a line to separate, meaningful categories seems desirable. The authors feel that this classification should reflect some aspect of possible gene action during one particular phase of the life of the bird. Because livability during the juvenile period was the earliest trait to be evaluated in this study, the homozygotes in each of the two-allele comparison groups were ranked on the basis of their relative favorable effect on juvenile livability into types 1 and 2 (Table 1, part C).

Two *B* alleles were present in each of lines H1, H3, H6, H8, H10, and H13; three alleles were present in line H2 (BRILES *et al.* 1957). Thus, in line H2 there

TABLE 1

*Juvenile livability—survival of mixed sexes to 150 days of age†*

| Line | Genotype | | No. fertile eggs | | Percent livability§ to 150 days | | Pooled over years | | Genotype × year interaction | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Part A. Homozygote 1 *vs.* heterozygote** | | | | | | | | | | |
| | Homo.1 | Hetero. | Homo.1 | Hetero. | Homo.1 | Hetero. | d.f. | $\chi^2$ | d.f. | $\chi^2$ |
| H1 | $B^1B^1$ | $B^1B^2$ | 195 | 388 | 65.6 | 67.5 | 1 | .0719 | 2 | .9210 |
| H2‡ | $B^3B^3$ | $B^3B^4$ | 37 | 73 | 62.2 | 84.9 | 1 | 1.6525 | 1 | .1318 |
| | $B^4B^4$ | $B^4B^5$ | 88 | 175 | 70.5 | 63.4 | 1 | .4363 | 2 | .3378 |
| | $B^5B^5$ | $B^4B^5$ | 91 | 129 | 44.0 | 52.7 | 1 | .8430 | 2 | 5.8372 |
| H3 | $B^7B^7$ | $B^6B^7$ | 202 | 322 | 50.0 | 48.8 | 1 | .0367 | 6 | 1.5412 |
| H6 | $B^8B^8$ | $B^8B^9$ | 334 | 606 | 45.5 | 51.5 | 1 | 1.5654 | 5 | 2.2787 |
| H8 | $B^{12}B^{12}$ | $B^{11}B^{12}$ | 623 | 738 | 38.0 | 50.4 | 1 | 11.5037** | 6 | .1130 |
| H10 | $B^{13}B^{13}$ | $B^{13}B^{14}$ | 308 | 589 | 54.5 | 45.7 | 1 | 3.2889 | 4 | .0738 |
| H13 | $B^{10}B^{10}$ | $B^8B^{10}$ | 161 | 312 | 59.6 | 53.8 | 1 | .6487 | 2 | 2.3871 |
| | Total | | 2039 | 3342 | 49.4§ | 53.4 | 9 | 20.0471* | 30 | 13.6216 |
| | Pooled over lines (homozygote 1 *vs.* heterozygote) | | | | | | 1 | .4019 | | |
| | Interaction (line × genotype) | | | | | | 8 | 19.6452* | | |
| **Part B. Homozygote 2 *vs.* heterozygote** | | | | | | | | | | |
| | Homo.2 | Hetero. | Homo.2 | Hetero. | Homo.2 | Hetero. | d.f. | $\chi^2$ | d.f. | $\chi^2$ |
| H1 | $B^2B^2$ | $B^1B^2$ | 237 | 430 | 58.6 | 64.2 | 1 | .7598 | 4 | 1.7142 |
| H2 | $B^4B^4$ | $B^3B^4$ | 130 | 166 | 56.2 | 78.9 | 1 | 5.4841* | 3 | .0000 |
| | $B^5B^5$ | $B^4B^5$ | 106 | 193 | 54.7 | 67.9 | 1 | 1.8728 | 3 | 1.8617 |
| | $B^4B^4$ | $B^4B^5$ | 84 | 122 | 57.1 | 66.4 | 1 | .6791 | 2 | 1.5681 |
| H3 | $B^6B^6$ | $B^6B^7$ | 119 | 239 | 42.9 | 45.6 | 1 | .1362 | 2 | 3.6261 |
| H6 | $B^9B^9$ | $B^8B^9$ | 318 | 591 | 43.1 | 52.1 | 1 | 3.4546 | 4 | 7.5261 |
| H8 | $B^{11}B^{11}$ | $B^{11}B^{12}$ | 136 | 251 | 49.3 | 62.2 | 1 | 2.5563 | 2 | 3.2152 |
| H10 | $B^{14}B^{14}$ | $B^{13}B^{14}$ | 501 | 782 | 44.1 | 46.4 | 1 | .3625 | 6 | 20.4618** |
| H13 | $B^8B^8$ | $B^8B^{10}$ | 194 | 345 | 48.5 | 52.2 | 1 | .3352 | 3 | .5341 |
| | Total | | 1825 | 3119 | 48.7 | 55.6 | 9 | 15.6406 | 29 | 40.5073 |
| | Pooled over lines (homozygote 2 *vs.* heterozygote) | | | | | | 1 | 10.3956** | | |
| | Interaction (line × genotype) | | | | | | 8 | 5.2450 | | |
| **Part C. Homozygote 1 *vs.* homozygote 2** | | | | | | | | | | |
| | Homo.1 | Homo.2 | Homo.1 | Homo.2 | Homo.1 | Homo.2 | d.f. | $\chi^2$ | d.f. | $\chi^2$ |
| H1 | $B^1B^1$ | $B^2B^2$ | 195 | 195 | 65.6 | 63.1 | 1 | .0996 | 2 | .0572 |
| H2 | $B^3B^3$ | $B^4B^4$ | 37 | 37 | 62.2 | 62.0 | 1 | .0000 | 1 | .0876 |
| | $B^4B^4$ | $B^5B^5$ | 88 | 88 | 70.5 | 53.4 | 1 | 2.0642 | 2 | .9632 |
| | $B^5B^5$ | $B^4B^4$ | 35 | 35 | 65.7 | 40.0 | 1 | 2.1890 | 1 | .6040 |
| H3 | $B^7B^7$ | $B^6B^6$ | 119 | 119 | 50.4 | 42.9 | 1 | .7296 | 2 | 4.2756 |
| H6 | $B^8B^8$ | $B^9B^9$ | 273 | 273 | 47.6 | 41.4 | 1 | 1.1892 | 3 | 4.2746 |
| H8 | $B^{12}B^{12}$ | $B^{11}B^{11}$ | 114 | 114 | 52.6 | 46.5 | 1 | .4336 | 1 | 1.0588 |
| H10 | $B^{13}B^{13}$ | $B^{14}B^{14}$ | 280 | 280 | 52.5 | 48.9 | 1 | .3520 | 3 | 8.1702* |
| H13 | $B^{10}B^{10}$ | $B^8B^8$ | 149 | 149 | 59.1 | 49.7 | 1 | 1.2098 | 1 | 2.5324 |
| | Total | | 1290 | 1290 | 56.8 | 49.2 | 9 | 8.2670 | 16 | 22.0236 |
| | Pooled over lines (homozygote 1 *vs.* homozygote 2) | | | | | | 1 | 7.0204** | | |
| | Interaction (line × genotype) | | | | | | 8 | 1.2466 | | |

† Males and females pooled; chicks hatched in 1954, 1955, and 1956 seasons.
‡ For purposes of this analysis, the three genotypic comparisons in line 2 are each treated statistically as if each represented a separate line.
§ Total number alive at 150 days/total number fertile eggs.
* Significant at the .05 level of probability.
** Significant at the .01 level of probability.

are three sets of comparisons possible between two opposite homozygous types and their combination heterozygotes—$B^3B^3$, $B^3B^4$, $B^4B^4$; $B^3B^3$, $B^3B^5$, $B^5B^5$; and $B^4B^4$, $B^4B^5$, $B^5B^5$. According to the classification scheme presented above, $B^3B^3$ of line H2 ranked as homozygote 1 when compared with each of the other two homozygotes, and $B^5B^5$ ranked as homozygote 1 when contrasted with $B^4B^4$.

Livability in the juvenile period for homozygote 1 *vs.* the heterozygote can be compared in part A of Table 1 for each of the lines. Only in line H8 did the survival value of the heterozygote (50.4 percent) exceed that of its contemporary homozygote 1 class (38.0 percent) more than would be expected by chance ($P < 0.01$). The second greatest difference in livability ($P < 0.08$) occurred in line H10 where the heterozygous class displayed a lower livability than homozygote 1 (45.7 *vs.* 54.5 percent). The chi-square analysis from Table 1, part A on the genotype × year interaction within lines and over all lines showed that the data were quite homogeneous, indicating that the relative livabilities of the two genotypic classes within lines were consistent over the three years covered by this study.

When the livability data for the nine comparisons were pooled and analyzed for line × genotype interaction, a significant chi-square (19.6452, d.f. = 8, $P = 0.02$) was obtained; this showed that the relative ranking of the two genotypes differed between lines. Inspection of the data reveals that the magnitude of the interaction chi-square is largely accounted for by the distinct heterozygote superiority observed in line H8 together with the failure of the other comparisons as a group to show distinct superiority of either of the genotypic classes.

Juvenile livability comparisons of homozygote 2 *vs.* the heterozygote for each of the lines are presented in Table 1, part B. In each of the nine comparisons, the heterozygote appeared superior to homozygote 2, but the difference was statistically significant ($P = 0.02$) only in one of the comparisons of line H2—$B^4B^4$ (56.2) vs. $B^3B^4$ (78.9). However, the livability values of the composite population (made up of all the lines) display a difference of considerable magnitude. There was a total of 1825 fertile eggs of homozygote 2 and 3119 fertile eggs of the heterozygous class with livability values of 48.7 and 55.6 percent, respectively—a difference significant beyond the 0.01 level of probability (chi-square = 10.3956, d.f. = 1). Furthermore the line × genotype interaction chi-square was very small (5.2450, d.f. = 8, $P = 0.73$), showing that the nine comparisons are homogeneous. The relative superiority of the heterozygote is especially significant in view of the consistency of the livability differences over years as shown by the nonsignificant genotype × year interaction for all lines except H10, where the difference in livability for the two groups (44.1 *vs.* 46.4 percent) was less than for any of the other lines.

In the homozygous *vs.* heterozygous comparisons presented in Table 1, there is a bias towards higher livability of the homozygotes of type 1 in part A and a bias towards lower livability of homozygotes of type 2 in part B. The bias in each case is the result of the inclusion of data from the heterozygous × heterozygous matings which formed the basis for the assignment of contrasting homozygotes to types 1 and 2 (Table 1, part C). In spite of including these data in the total

matings over the seven lines, homozygotes of type 1 did not have higher livability than the heterozygotes (Table 1, part A); on the other hand, homozygotes of type 2 were distinctly lower in livability than the heterozygotes ($P < 0.01$); Table 1, part B). This lack of symmetry in the separate comparison of the heterozygotes with the two classes of homozygotes, when the bias would be expected to be of similar magnitude in each direction, lends credence to the probable existence of superior livability of the heterozygotes over their contemporary sibs of homozygote type 2.

Juvenile livability data for contrasting homozygotes are presented in Table 1, part C. The difference in survival value of homozygote 1 *vs.* homozygote 2 is not significant for any of the individual lines. It should, however, be emphasized that the data for the homozygote 1 *vs.* homozygote 2 comparisons were necessarily limited to the progeny from the heterozygous × heterozygous matings. As a result these classes usually consisted of fewer progeny than in the other comparisons, which also frequently included offspring from heterozygous × homozygous matings (Table 1, parts A and B).

In observing the relative survival of the two homozygous types over the nine comparison groups, it must be recalled that the homozygote having the highest livability was designated type 1, so that pooling over the lines would be expected to result in a spurious livability difference for homozygote 1 *vs.* homozygote 2. Therefore, the highly significant chi-square obtained for the pooled data (7.0204, d.f. = 1) cannot be interpreted as demonstrating that one homozygote is significantly higher in livability than the other. At the same time, it does not rule out the possibility that an over-all difference may exist.

The data presented in Table 1 have shown in line H8 and in one of the comparison classes of line H2 ($B^4B^4$ vs. $B^3B^4$) that one of the homozygous types was inferior to the heterozygote. Furthermore, the homozygote assigned as type 2 solely on the basis of its having lower average livability than its opposite homozygote (type 1), ranked below the heterozygote in each of the nine comparison groups (Table 1, part B). Viewed as a whole, these data are indicative of dominance, with overdominance a distinct possibility in line H6, H8, and between certain alleles in line H2 ($B^3$ vs. $B^4$ and $B^4$ vs. $B^5$).

When the homozygotes were pooled over all lines and compared with the pooled heterozygotes, the respective juvenile livabilities were 49.0 percent (1895 survivors/ 3864 fertile eggs) and 54.2 percent (2095 survivors/ 3868 fertile eggs). This difference in livability is highly significant (chi-square = 9.8056, d.f. = 1) and discloses that among the birds tested there was a distinct tendency for at least one of the homozygotes to rank below the heterozygote.

*Adult livability:* The combined livability of males and females for the period between housing (approximately 150 days) to 450 days of age is presented in Table 2, parts A, B, and C. In line H1, where the three genotypic classes survived about equally well during the juvenile period, the adult livability was quite low for homozygote 1 (Table 2, part A). Only 12.8 percent of the total adults of this type ($B^1B^1$) survived the 450-day age period; whereas 47.4 percent of the corresponding sibs belonging to the heterozygous class ($B^1B^2$) survived this period.

TABLE 2

*Adult livability—survival of mixed sexes from 150 to 450 days of age†*

| Line | Genotype | | No. birds housed | | Percent livability§ 150 to 450 days | | Pooled over years | | Genotype × year interaction | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Part A. Homozygote 1 *vs.* heterozygote** | | | | | | | | | | |
| | Homo.1 | Hetero. | Homo.1 | Hetero. | Homo.1 | Hetero. | d.f. | $\chi^2$ | d.f. | $\chi^2$ |
| H1 | $B^1B^1$ | $B^1B^2$ | 86 | 171 | 12.8 | 47.4 | 1 | 29.8068** | 2 | 8.2592* |
| H2‡ | $B^3B^3$ | $B^3B^4$ | 11 | 24 | 81.8 | 87.5 | 1 | .1717 | 0 | ..... |
| | $B^3B^3$ | $B^3B^5$ | 33 | 72 | 33.3 | 47.2 | 1 | 1.7897 | 1 | .7298 |
| | $B^5B^5$ | $B^4B^5$ | 34 | 59 | 79.4 | 78.0 | 1 | .0245 | 2 | .5338 |
| H3 | $B^7B^7$ | $B^6B^7$ | 80 | 125 | 48.8 | 54.4 | 1 | .6440 | 6 | 5.9591 |
| H6 | $B^8B^8$ | $B^8B^9$ | 103 | 196 | 57.3 | 53.6 | 1 | .3737 | 4 | 2.5808 |
| H8 | $B^{12}B^{12}$ | $B^{11}B^{12}$ | 178 | 249 | 25.8 | 28.5 | 1 | .3549 | 5 | 2.6826 |
| H10 | $B^{13}B^{13}$ | $B^{13}B^{14}$ | 96 | 177 | 43.6 | 45.2 | 1 | .4113 | 4 | 2.1883 |
| H13 | $B^{10}B^{10}$ | $B^8B^{10}$ | 40 | 89 | 60.0 | 60.7 | 1 | .0059 | 2 | 1.6782 |
| | Total | | 661 | 1162 | 40.5§ | 48.3 | 9 | 33.5825** | 26 | 24.6118 |
| | Pooled over lines (homozygote 1 *vs.* heterozygote) | | | | | | 1 | 10.1603** | | |
| | Interaction (line × genotype) | | | | | | 8 | 23.4222** | | |
| **Part B. Homozygote 2 *vs.* heterozygote** | | | | | | | | | | |
| | Homo.2 | Hetero. | Homo.2 | Hetero. | Homo.2 | Hetero. | d.f. | $\chi^2$ | d.f. | $\chi^2$ |
| H1 | $B^2B^2$ | $B^1B^2$ | 98 | 184 | 61.2 | 55.6 | 1 | .6932 | 4 | .7041 |
| H2 | $B^4B^4$ | $B^3B^4$ | 49 | 74 | 32.7 | 50.0 | 1 | 3.5980 | 1 | .0000 |
| | $B^5B^5$ | $B^3B^5$ | 40 | 82 | 30.0 | 47.5 | 1 | 3.3778 | 2 | .0000 |
| | $B^4B^4$ | $B^4B^5$ | 33 | 56 | 36.4 | 58.9 | 1 | 4.2214* | 2 | .0000 |
| H3 | $B^6B^6$ | $B^6B^7$ | 46 | 94 | 58.7 | 55.3 | 1 | .1315 | 2 | 7.5297* |
| H6 | $B^9B^9$ | $B^8B^9$ | 102 | 214 | 53.9 | 57.0 | 1 | .1527 | 4 | 7.7496 |
| H8 | $B^{11}B^{11}$ | $B^{11}B^{12}$ | 38 | 96 | 47.4 | 31.3 | 1 | 3.0939 | 1 | .0000 |
| H10 | $B^{14}B^{14}$ | $B^{13}B^{14}$ | 142 | 206 | 38.7 | 47.1 | 1 | 2.4275 | 6 | 10.7534 |
| H13 | $B^8B^8$ | $B^8B^{10}$ | 60 | 99 | 50.0 | 60.6 | 1 | 1.7435 | 3 | 2.5825 |
| | Total | | 608 | 1105 | 46.4 | 50.5 | 9 | 19.4395* | 25 | 29.3193 |
| | Pooled over lines (homozygote 2 *vs.* heterozygote) | | | | | | 1 | 2.6445 | | |
| | Interaction (line × genotype) | | | | | | 8 | 16.7950* | | |
| **Part C. Homozygote 1 *vs.* homozygote 2** | | | | | | | | | | |
| | Homo.1 | Homo.2 | Homo.1 | Homo.2 | Homo.1 | Homo.2 | d.f. | $\chi^2$ | d.f. | $\chi^2$ |
| H1 | $B^1B^1$ | $B^2B^2$ | 86 | 84 | 12.8 | 58.3 | 1 | 38.3822** | 2 | .9167 |
| H2 | $B^3B^3$ | $B^4B^4$ | 11 | 10 | 81.8 | 80.0 | 1 | .0122 | 0 | ..... |
| | $B^3B^3$ | $B^5B^5$ | 33 | 29 | 33.3 | 31.0 | 1 | .0265 | 1 | .0069 |
| | $B^5B^5$ | $B^4B^4$ | 18 | 9 | 83.3 | 66.7 | 1 | .9642 | 1 | .0000 |
| H3 | $B^7B^7$ | $B^6B^6$ | 51 | 46 | 52.9 | 58.7 | 1 | .3282 | 2 | 1.4466 |
| H6 | $B^8B^8$ | $B^9B^9$ | 92 | 76 | 64.1 | 53.9 | 1 | 1.7588 | 3 | 3.5859 |
| H8 | $B^{12}B^{12}$ | $B^{11}B^{11}$ | 44 | 38 | 36.4 | 47.4 | 1 | .9777 | 1 | .0000 |
| H10 | $B^{13}B^{13}$ | $B^{14}B^{14}$ | 96 | 97 | 42.7 | 44.3 | 1 | .0538 | 3 | 2.5476 |
| H13 | $B^{10}B^{10}$ | $B^8B^8$ | 31 | 43 | 61.3 | 53.5 | 1 | .4431 | 1 | .3254 |
| | Total | | 462 | 432 | 44.4 | 53.5 | 9 | 42.9467** | 14 | 8.8291 |
| | Pooled over lines (homozygote 1 *vs.* homozygote 2) | | | | | | 1 | 7.3878** | | |
| | Interaction (line × genotype) | | | | | | 8 | 35.5589** | | |

† Males and females pooled; adults hatched in 1954, 1955, and 1956 seasons.

‡ For purposes of this analysis, the three genotypic comparisons in line 2 are each treated statistically as if each represented a separate line.

§ Total number alive at 450 days/total number housed at 150 days.

* Significant at the .05 level of probability.

** Significant at the .01 level of probability.

On the other hand, homozygote 2 ($B^2B^2$) with livability value of 61.2 percent did not differ significantly from the heterozygote with a livability value of 55.6 percent (Table 2, part B). Here we have an example of two homozygous classes which show no difference in livability during the juvenile period yet are markedly different in livability during the adult period. As far as general fecundity is concerned, the homozygous genotype $B^1B^1$ is functionally almost a complete lethal. Numerous matings involving $B^1B^1$ pullets have been made, but only two have produced progeny; furthermore, not a single chick has resulted from five years of attempting to obtain progeny from several $B^1B^1 \times B^1B^1$ matings. There is some evidence that the livability of $B^1B^1$ homozygotes may be especially subject to yearly environmental fluctuations. This is indicated by a significant genotype × year interaction for the $B^1B^1$ vs. $B^1B^2$ livability comparisons (Table 2, part A). The survival of all line H1 adults for the years 1954, 1955, and 1956 was 40.7, 26.8, and 66.4 percent, respectively, while the $B^1B^1$ adults had corresponding survival values of 0.0, 2.0, and 35.7 percent.

In line H2 the genotypic classes consisted of relatively fewer individuals per class because the progeny each year were necessarily distributed over six genotypes as a result of the three alleles present in this population; whereas the progeny of each of the other lines segregated into only three genotypes as a result of two alleles being present. This is especially important in the relatively smaller adult population. In spite of this unfavorable sample size, the adult livability of $B^4B^5$ class was significantly higher ($P = 0.05$) than the $B^4B^4$ class (homozygote 2)—the values being 58.9 *vs.* 36.4 percent, respectively (Table 2, part B). Also, the heterozygotes $B^3B^4$ and $B^3B^5$ outlived their respective homozygous 2 types, $B^4B^4$ and $B^5B^5$, at values closely approaching significance, 50.0 *vs.* 32.7 ($P = 0.06$) and 47.6 *vs.* 30.0 ($P = 0.07$), respectively (Table 2, part B). At the same time, the very limited data for the three sets of contemporary homozygote comparisons indicate about equal relative survival for each, except possibly for $B^4B^4$ vs. $B^5B^5$ (Table 2, part C).

None of the three genotypic classes in the New Hampshire line H3 differed significantly in adult livability; however, homozygote 2 ($B^6B^6$) ranked above the other homozygote ($B^7B^7$) and the heterozygote ($B^6B^7$) which in turn ranked above $B^7B^7$, suggesting a favorable effect on livability for the $B^6$ allele.

Before observing the data for line H6 and H13, it should be restated that these two lines were derived from the same Rhode Island Red breeding flock and that one allele, $B^8$, is held in common by these two inbred lines (Briles *et al.* 1957). Although differences in adult livability between genotypes within these lines are not significant statistically, it is perhaps pertinent to point out that in each line the heterozygote ranked above homozygote 2 ($B^8B^9$ in H6 and $B^8B^8$ in H13, Table 2, part B) and that homozygote 1 ($B^8B^8$ in H6 and $B^{10}B^{10}$ in H13) ranked above homozygote 2 (Table 2, part C). In line H6 the livability of homozygote 1 ($B^8B^8$) was higher than the heterozygote ($B^8B^9$); in line H13 the livability of the homozygote 1 ($B^{10}B^{10}$) was equal to that of the heterozygote ($B^8B^{10}$). Thus, $B^8$ appears to be superior to $B^9$ in line H6 but inferior to $B^{10}$ in line H13—the three

alleles ranking in the order $B^{10}$, $B^{8}$, $B^{9}$ according to their favorable effects on adult livability.

In Leghorn lines H8 and H10 there were no statistically significant differences in adult livability between any of the genotypic comparisons. However, in line H8 the $B^{11}$ allele did show consistently a favorable effect.

Pooling the adult livability data for all lines resulted in significant differences in livability for two of the three comparison classes—homozygote 1 *vs.* heterozygote and homozygote 1 *vs.* homozygote 2 (Table 2, parts A and C). The exceptionally low livability of homozygote 1 of line H1 ($B^{1}B^{1}$) contributes heavily toward the pooled differences in both comparison groups and also largely accounts for the significant line × genotype interaction present for these two comparisons. The genotype × year interaction chi-squares for the seven lines as composite comparison groups were well within chance expectation, although as individual line comparisons homozygote 1 *vs.* heterozygote in H1 and homozygote 2 *vs.* heterozygote in H3 showed interactions significant at the 0.05 probability level (Table 2, parts A and B).

The apparent general superiority of the heterozygotes may stem from the somewhat chance assignment of the adult homozygotes to types 1 and 2 on the prior criterion of relative juvenile livability. If the individual homozygotes whose *adult* livability compared most favorably with their corresponding heterozygotes are compared as a group, the relative livability values of the "best" adult homozygotes *vs.* their corresponding heterozygotes are 55.5 and 53.2 percent, respectively, with line × genotype interaction being nonsignificant. This means that in general one of the homozygotes in each of the nine comparison groups was about equal in livability to the heterozygotes. On the other hand, when the opposite homozygotes (those comparing least favorably with their respective heterozygotes) are compared as a group to the heterozygotes the livability values are 35.8 and 47.6 percent, respectively—a difference significant beyond the 0.01 level of probability (chi-square = 27.2630, d.f. = 1). Even if line H1, with its extremely low livability homozygote, is eliminated from consideration, the difference is still significant beyond the 0.01 level (chi-square = 13.7151, d.f. = 1) with no significant line × genotype interaction; yet only one of the eight comparisons, $B^{4}B^{4}$ vs. $B^{4}B^{5}$ of line H2 (Table 2, part A), showed a significant difference when individually considered.

Pooling extremes as above would be expected to create artificial differences, but the wide divergence between the two sets of comparisons does suggest a general manifestation of dominance by the various two-allele comparison groups. When the homozygotes were compared directly (Table 2, part C) a significant difference occurred only in line H1, although the highest ranking of the two homozygotes also compared more favorable with their respective heterozygotes in eight of the nine two-allele comparison groups, the exception being line H10.

When the homozygotes were pooled over all lines and compared with the heterozygotes, the respective adult livabilities were 42.3 percent (543 survivors/ 1283 housed) and 48.0 percent (632 survivors/ 1317 housed). This difference in livability is highly significant (chi-square = 8.4128, d.f. = 1) and shows that,

even though one of the homozygotes in each two-allele comparison group may be about equivalent to the heterozygote, the other homozygote on the average must be sufficiently inferior to lower the pooled homozygote value below that of the heterozygotes.

Adult livability data for females is presented in Table 3; these data include the females already considered in the combined males and females of Table 2 plus other contemporary nonsisters. The effect of the *B* genotypes on livability of line H1 females resembles closely that for mixed sexes; the livability of the heterozygote and homozygote 2 are high and approximately equal (89.6 and 87.0 percent, respectively), and homozygote 1 is again decidedly inferior (31.0 percent). In line H2 (where the three sets of genotypes have been pooled because of small numbers) and in line H3, the heterozygote and homozygote 2 classes are about equal and rank above homozygote 1 in livability, although the differences are not statistically significant. Lines H6 and H13, derived from the same original Rhode Island Red population, show essentially the same livability for each of the three genotypic classes. In line H8, as in the data where both sexes were combined, homozygote 2 ($B^{11}B^{11}$) outlived birds of the other two genotypes; however, the 17 birds of this type hardly constitute an adequate sample. Homozygote 1 females ($B^{13}B^{13}$) of line H10 showed higher livability than either the heterozygote ($B^{13}B^{14}$) or homozygote 2 ($B^{14}B^{14}$); when the data from the males were included, the heterozygote ranked above both homozygotes in livability (Table 2). In general the relative livability differences shown for each line by the adult females resemble rather closely those resulting from the combined adult males and females, line H10 possibly constituting an exception.

Comparing adult female livability values of both types of homozygotes pooled with that of the total heterozygotes resulted in survival values of 68.5 (518/756) and 72.8 (466/640) percent, respectively (chi-square = 3.0384, P = 0.09).

*Relative effects in juvenile and adult periods:* In four of the seven inbred lines

TABLE 3

*Adult female livability†*

| Line | Homozygote 1 Number housed | Homozygote 1 Percent livability | Heterozygote Number housed | Heterozygote Percent livability | Homozygote 2 Number housed | Homozygote 2 Percent livability | Pooled over years d.f. | Pooled over years $\chi^2$ | Genotype × year interaction d.f. | Genotype × year interaction $\chi^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| H1 | 29 | 31.0 | 77 | 89.6 | 77 | 87.0 | 2 | 49.0775** | 4 | 5.5884 |
| H2 | 89 | 80.9 | 117 | 88.0 | 29 | 89.7 | 2 | 2.5054 | 4 | 4.7892 |
| H3 | 84 | 56.0 | 79 | 70.9 | 43 | 67.4 | 2 | 4.4916 | 4 | 9.0176 |
| H6 | 48 | 72.9 | 98 | 73.5 | 55 | 74.5 | 2 | .0348 | 4 | 2.0884 |
| H8 | 94 | 50.0 | 98 | 44.9 | 17 | 70.6 | 2 | 3.8252 | 4 | 1.7576 |
| H10 | 56 | 69.6 | 104 | 64.4 | 67 | 56.7 | 2 | 2.3220 | 4 | 4.2679 |
| H13 | 29 | 86.2 | 67 | 82.1 | 39 | 79.5 | 2 | .4808 | 4 | 4.2456 |
| Total | 429 | 63.9‡ | 640 | 72.8 | 327 | 74.6 | 14 | 62.7373** | 28 | 31.7547 |
| | | | Genotypes pooled over lines | | | | 2 | 13.3856** | | |
| | | | Interaction (line × genotype) | | | | 12 | 49.3517** | | |

** Significant at the .01 level of probability.
† These data include the females already considered in mixed sexes of Table 2 plus other contemporary nonsisters.
‡ Total number alive (at 450 days)/total number housed (at 150 days).

the relative rank of homozygotes 1 and 2 reverse in the juvenile and adult mortality phases. By comparing the relative average livabilities in part C of Tables 1 and 2, it can be seen that the order of rank of the homozygotes is reversed in Leghorn lines H1, H8, and H10 and in New Hampshire line H3. The order of relative rank of the two homozygotes was the same in both livability periods for Leghorn line H2, having three alleles, and the Rhode Island Red lines H6 and H13, derived from the same original breeding flock. A chi-square analysis was made on the pooled data from the seven lines to determine the degree of interaction of the two homozygous types in the juvenile and adult phases (Table 4). When the differences between the two homozygous types were analyzed separately for the juvenile and adult phases and the chi-squares pooled, a value of 14.4082 with two degrees of freedom was obtained ($P < 0.01$). On the other hand, when the relative livabilities of the two homozygous types for all lines over the entire period from fertilization to 450 days were analyzed, a non-significant chi-square value of 0.3972 with one degree of freedom resulted. The difference between these two chi-squares (14.0110, d.f. = 1) gives the interaction of the effects of the homozygotes during the juvenile and adult phases and is significant beyond the 0.01 level of probability. This interaction is primarily due to line H1, where homozygote 2 was distinctly superior for adult livability. A similar chi-square analysis leaving out the data from line H1 still showed interaction to be present but only at the 0.10 level of probability. Thus, chi-square analysis reveals that a distinct shift in relative effectiveness of the homozygous types in the two livability periods occurred in line H1 and that the reversal in the relative livability of the opposite homozygous types observed in H3, H8, and H10 may be real in one or more of the lines.

*Relative response of genotypes to year differences:* The general consistency of the various genotypic comparisons in time has been demonstrated in Tables 1, 2, and 3 by low chi-square values for the genotype × year interactions in the totaled lines and within lines except for certain comparisons occurring in H1 (Table 2, part A), H3 (Table 2, part B), and H10 (Table 1, parts B and C).

The effects of the environments in the different years on each of the three genotypic groupings—homozygote 1, heterozygote, and homozygote 2—were determined by chi-square homogeneity analysis computed separately within the

TABLE 4

*Interaction of homozygotes 1 and 2 in juvenile and adult livability periods*

| | Percent livability | | d.f. | $\chi^2$ |
|---|---|---|---|---|
| | Homozygote 1 | Homozygote 2 | | |
| Total homozygotes | | | 2 | 14.4082** |
| Juvenile (0–150 days) | 56.8 | 49.2 | | |
| Adult (150–450 days) | 44.4 | 53.5 | | |
| Pooled homozygotes | | | 1 | .3972 |
| (Juvenile and adult phases) | 25.2 | 26.3 | | |
| Interaction of homozygotes (Juvenile and adult phases) | | | 1 | 14.0110** |

** Significant at the .01 level of probability.

juvenile and adult livability periods. To illustrate the procedure used, the analysis of the effect of year on juvenile livability of homozygote 1 is presented in Table 5. It is evident from the analysis that birds of $B^3B^3$, $B^{10}B^{10}$, and $B^{12}B^{12}$ genotypes in themselves differed significantly in juvenile livability from year to year. When all of the lines were totaled and the pooled effect of the three years removed, the genotype × year interaction was not significant, showing that homozygotes of type 1 from the various lines responded in similar manner to the environmental differences affecting livability from one year to the next.

As is evident from Table 5, juvenile livability in general increased over the three years, reflecting improved rearing conditions to which the birds were uniformly exposed (intermingled). The average juvenile livability (mean of homozygote 1, homozygote 2, and heterozygotes) for each of the three successive years was 40.4, 50.8, and 57.9 percent, respectively. Corresponding average livabilities for each year of the adult period were 47.5, 26.2, and 67.9 percent; the low livability during the second year was largely due to a nonspecific respiratory infection.

The genotype × year interaction chi-square of 0.5809, d.f. = 12, from Table 5 and similarly derived values for the five other genotype-livability groupings are summarized in Table 6. Homozygote 1 does not interact with years in the juvenile period ($P < 0.99$) but does show a significant interaction for the adult

TABLE 5

*Chi-square analysis of effect of year on juvenile livability of homozygote 1*

| Line | Genotype | Percent livability within years 1954 | 1955 | 1956 | d.f. | $\chi^2$ |
|---|---|---|---|---|---|---|
| H1 | $B^1B^1$ | 42.1 | 64.6 | 71.1 | 2 | 2.0739 |
| H2 | $B^2B^2$ | 49.1 | 100.0 | 71.4 | 2 | 7.2339* |
| | $B^5B^5$ | (Three years not available) | | | | |
| H3 | $B^7B^7$ | 43.5 | 41.0 | 62.0 | 2 | 3.7027 |
| H6 | $B^6B^6$ | 36.6 | 43.9 | 51.5 | 2 | 3.1098 |
| H8 | $B^{12}B^{12}$ | 26.9 | 32.1 | 46.7 | 2 | 11.0941** |
| H10 | $B^{13}B^{13}$ | 43.3 | 59.0 | 60.4 | 2 | 3.3157 |
| H13 | $B^{10}B^{10}$ | 66.7 | 39.7 | 71.4 | 2 | 6.1298* |
| Total | | | | | 14 | 36.7499** |
| Pooled over lines | | 36.3 | 46.6 | 59.2 | 2 | 36.1690** |
| Interaction (genotype × year) | | | | | 12 | 0.5809 |

* Significant at .05 level of probability.
** Significant at .01 level of probability.

TABLE 6

*Genotype × year interactions for livability*

| Genotype | Juvenile livability d.f. | $\chi^2$ | P | Adult livability d.f. | $\chi^2$ | P |
|---|---|---|---|---|---|---|
| Homozygote 1 | 12 | 0.5809 | >.99 | 10 | 26.6661 | <.01 |
| Heterozygote | 16 | 32.9357 | <.01 | 12 | 33.0369 | <.01 |
| Homozygote 2 | 14 | 32.7690 | <.01 | 10 | 6.7215 | >.75 |

period ($P < 0.01$). Homozygote 2 reacts in an opposite manner—strong interaction in the juvenile period ($P < 0.01$) but no interaction in the adult period ($P > 0.75$). Thus, the homozygous types which the preceding data (Tables 1 and 2) indicated were in general most likely to be superior in the juvenile and adult periods—homozygotes 1 and 2, respectively—do not interact with years during the phase when they most favorably influenced livability. The heterozygotes interacted with years during both the juvenile and adult periods ($P < 0.01$).

*Egg production:* The average percent hen-day production was used as an index of egg production and was calculated for each of the genotypic categories within each line (Table 7). The percent hen-day production as used here represents

TABLE 7

*Egg production of inbreds on basis of* B *blood group genotypes*

| | | Percent hen-day egg production | | | | | | Analysis of variance | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Homozygote 1 | | Heterozygote | | Homozygote 2 | | Blood type within families | | Error† | |
| Line | Year | No. of pullets | Percent production | No. of pullets | Percent production | No. of pullets | Percent production | d.f. | Mean square | d.f. | Mean square |
| H1 | 1955 | 1 | 0.0 | 14 | 42.1 | 13 | 41.5 | 5 | 1,363.34 | 17 | 495.92 |
| | 1956 | 6 | 5.8 | 21 | 29.8 | 14 | 32.9 | 13 | 454.01 | 18 | 312.06 |
| | 1957 | 18 | 24.9 | 28 | 42.7 | 27 | 39.9 | 19 | 1,159.89** | 41 | 116.07 |
| | Avg.‡ | | 10.2 | | 38.2 | | 38.1 | 37 | 939.37** | 76 | 247.45 |
| H2 | 1955 | 7 | 50.7 | 12 | 38.8 | 4 | 44.1 | 9 | 260.05* | 18 | 77.97 |
| | 1956 | 35 | 21.7 | 20 | 33.1 | 13 | 24.6 | 10 | 281.44 | 57 | 364.76 |
| | 1957 | 26 | 31.4 | 31 | 35.3 | 6 | 34.6 | 3 | 33.67 | 52 | 318.13 |
| | Avg. | | 34.6 | | 35.7 | | 34.4 | 22 | 238.86 | 127 | 305.02 |
| H3 | 1955 | 17 | 19.3 | 25 | 23.7 | 6 | 31.2 | 8 | 1,389.30** | 28 | 193.77 |
| | 1956 | 17 | 16.3 | 10 | 26.9 | 7 | 13.2 | 5 | 334.77 | 18 | 431.38 |
| | 1957 | 30 | 23.5 | 21 | 27.4 | 22 | 30.5 | 9 | 63.57 | 53 | 223.17 |
| | Avg. | | 19.7 | | 26.0 | | 25.0 | 22 | 607.30** | 99 | 252.71 |
| H6 | 1955 | 6 | 49.4 | 15 | 49.5 | 6 | 46.5 | 5 | 599.48 | 19 | 452.17 |
| | 1956 | 10 | 35.2 | 25 | 36.6 | 12 | 24.5 | 5 | 382.88 | 31 | 495.54 |
| | 1957 | 19 | 33.7 | 23 | 30.3 | 18 | 36.4 | 8 | 237.27 | 45 | 301.21 |
| | Avg. | | 39.4 | | 38.8 | | 35.8 | 18 | 378.33 | 95 | 394.82 |
| H8 | 1955 | 15 | 53.6 | 12 | 72.9 | 2 | 51.4 | 6 | 1,581.25** | 16 | 301.01 |
| | 1956 | 20 | 40.1 | 37 | 30.4 | 7 | 32.6 | 14 | 720.19 | 38 | 409.30 |
| | 1957 | 41 | 39.1 | 28 | 38.7 | 10 | 34.7 | 10 | 332.94 | 58 | 377.35 |
| | Avg. | | 44.3 | | 47.3 | | 39.6 | 30 | 763.32** | 112 | 377.28 |
| H10 | 1955 | 8 | 60.8 | 18 | 67.9 | 12 | 59.2 | 6 | 163.56 | 26 | 383.70 |
| | 1956 | 19 | 52.1 | 36 | 44.1 | 17 | 27.5 | 12 | 604.24 | 41 | 382.21 |
| | 1957 | 20 | 53.5 | 21 | 53.3 | 20 | 50.9 | 13 | 254.79 | 38 | 425.19 |
| | Avg. | | 55.5 | | 55.1 | | 45.9 | 31 | 372.41 | 105 | 398.14 |
| H13 | 1955 | 4 | 35.3 | 17 | 29.0 | 3 | 40.5 | 3 | 560.44* | 14 | 141.18 |
| | 1956 | 6 | 29.1 | 30 | 23.3 | 12 | 26.0 | 7 | 399.14 | 30 | 349.42 |
| | 1957 | 17 | 30.0 | 19 | 32.6 | 19 | 15.4 | 6 | 380.78 | 44 | 259.05 |
| | Avg. | | 31.5 | | 28.3 | | 27.3 | 16 | 422.50 | 88 | 271.10 |

* Significant at the .05 level of probability.
** Significant at the .01 level of probability.
† Error represents mean square between sisters within blood type categories.
‡ Mean of yearly averages.

the number of eggs laid by pullets of a particular genotype expressed as a percentage of the total number of hen-days credited to that particular type. The data on egg production were obtained from the same females as those used for the adult female mortality study (Table 3) except that those dying between housing and the beginning of the trap period are not included.

Using the analysis of variance, sire and dam sources of variation were removed and blood type variance within sires and dams was compared to the error variance (Table 7). This analysis disclosed highly significant differences in egg production between *B* blood group genotypes in lines H1, H3, and H8. In each of these lines, the percent hen-day egg production of the heterozygote and one of the homozygotes was very nearly equal and both were superior to that of the other homozygote.

Considering the entire group of seven lines as a composite population, a pooled analysis of variance disclosed that the over-all effect of blood group genotype on egg production was significant at the 0.01 level of probability (Table 8). The average percent hen-day egg production for the classes pooled over all lines was 33.5, 38.5, and 35.1 for homozygote 1, heterozygote, and homozygote 2, respectively. The apparent over-all superiority of the heterozygous class undoubtedly stems largely from the general association of one of the homozygotes in each two-allele comparison group with a lower rate of egg production.

The ranking of genotypes within lines as to their relative influence on egg production followed in general that for adult female livability; only lines H6 and H8 showed any tendency toward reversal, and these are of doubtful meaningfulness.

## CONCLUSIONS AND DISCUSSION

In evaluating quantitative polygenic traits like livability and egg production we are dealing at most with only the periphery of multitudinous physiological processes as they are integrated over the life span of the individuals comprising the population under study. Consequently, the magnitude of the differences in the measurable end effects of various genotypes at a particular locus would usually be expected to be small and to require exceptionally large samples to establish their existence with desirable levels of confidence. Nevertheless, such differences would be of biological importance, especially on a population level.

TABLE 8

*Analysis of variance of egg production*

| Source of variation | d.f. | Mean square |
|---|---|---|
| Lines | 6 | 871.45** |
| Years | 2 | 1,071.49** |
| Year × line interaction | 12 | 93.90 |
| Blood group genotypes within each line | 14 | 139.71** |
| Blood group genotypes within each line × year interaction | 28 | 44.03 |

** Significant at the .01 level of probability.

Thus, some of the performance differences between genotypes, even though they are below that required for the generally accepted 0.05 probability level of significance, may be indicative of important gene action effects when considered in conjunction with other genotypic groupings involving the same alleles.

Viewing the present data broadly in this manner reveals some rather definite relationships. In each of the seven lines some degree of dominance was expressed by the *B* locus classifications for one or more of the economic traits studied (juvenile livability, adult livability of mixed sexes, adult livability of females, and egg production). A brief summary statement regarding the relative genotypic effects in each line follows:

*Line H1:* The three genotypes are essentially equal with regard to juvenile livability, but during the adult phase a high degree of dominance was exhibited by the $B^2$ allele—the $B^1B^1$ homozygote being decidedly inferior for livability and egg production (Tables 2, 3, and 7).

*Line H2:* In this line, where three alleles resulted in three sets of contemporary heterozygous-homozygous groupings, $B^3$ vs. $B^4$ comparisons exhibit overdominance in both the juvenile and adult livability periods (Tables 1 and 2); $B^3$ vs. $B^5$ comparisons exhibit additive superiority of $B^3$ in the juvenile livability period (Table 1) but show evidence of overdominance for adult livability (Table 2); and $B^4$ vs. $B^5$ comparisons exhibit doubtful overdominance in the juvenile period (Table 1) and rather distinct dominance of $B^5$ during the adult phase (Table 2).

*Line H3:* During the adult period $B^6$ shows a dominant superiority over $B^7$ for livability (Tables 2 and 3) and egg production (Table 7), while in the juvenile period a weak but consistent favorable effect is associated with $B^7$.

*Line H6:* Overdominance is shown for juvenile livability (Table 1), but $B^8$ exerts a consistent additive effect over $B^9$ for adult livability of mixed sexes (Table 2) and possibly for egg production (Table 7). Neither allele exhibits any measurable influence on livability of adult females (Table 3).

*Line H8:* Overdominance is shown for juvenile livability and possibly for egg production, but $B^{11}B^{11}$ birds show higher adult livability than those with $B^{11}B^{12}$ or $B^{12}B^{12}$ genotypes (Tables 2 and 3).

*Line H10:* Allele $B^{13}$ shows evidence of being dominant over $B^{14}$ for juvenile livability (Table 1), adult female livability (Table 3), and egg production (Table 7), with a weak indication of overdominance for adult livability of mixed sexes (Table 2).

*Line H13:* $B^{10}$ displays additive superiority over $B^8$ for juvenile and adult female livability (Tables 1 and 3) and shows dominance for adult livability of mixed sexes (Table 2).

From the above general considerations, it appears that the relationships between the two alleles in each of the lines (or comparison groups) reveal the superiority of one allele over the other for one or more of the characters measured. Considering the lines as a whole, the preference for one allele begins at the level of additiveness and increases through various degrees of dominance to complete dominance, and in several instances the alleles appear to interact so as to result in overdominance.

Some indication of one type of gene action that may be involved in the over-all expression of overdominance is afforded by the data and analysis presented in Table 4. The relative mean livability of the two homozygous types was reversed for the juvenile and adult periods. This is shown by a significant interaction chi-square for pooled homozygotes of type 1 *vs.* pooled homozygotes of type 2 in the two livability periods. Although the interaction was primarily due to line H1, other populations exhibited this reversal to some degree (lines H3, H8, and H10). Reversal of superiority of homozygotes for the two adult traits, livability and egg production, was indicated only in line H8 where extreme disproportionality of subclass numbers cast considerable doubt on its being real.

The phenomenon of alternation of maximum effectiveness of the two homozygous types, either in time for the same or different traits or simultaneously for different traits, may be an important mechanism by which the polymorphism at the *B* locus is maintained. A somewhat analogous situation involving a pair of testis color alleles in the moth *Ephestia kühniella* has been reported by CASPARI (1950, 1951). A dominant allele, *Rt*, produces brown pigment in the testis; the recessive allele, *rt*, produces a red pigment. With regard to viability, the heterozygote is equal or superior to the homozygous recessive genotype, *rt rt*, and the latter is superior to the homozygous dominant genotype, *Rt Rt*. As regards mating activity, the heterozygote is at least equal to *Rt Rt* which is superior to *rt rt*. In this species, polymorphism with respect to testis color exists on both the European and American continents and appears to result primarily from an alternate superiority of the two alleles for viability and mating activity.

The extent to which alternate superiority of contrasting *B* alleles accounts for the apparent overdominance can only be speculated on at this time. The overdominance indicated in this study for the juvenile period could result from an averaging of the effects of alternate superiority of the *B* alleles during periods covering the various developmental phases. The incubation period and the time from hatching to sexual maturity certainly cover a metabolic spectrum sufficiently broad to afford opportunity for expression of alternate superiority of alleles. In a previous study of the B blood group system BRILES and KRUEGER (1955) found in three related inbred lines that both hatchability and chick livability (to nine weeks) were higher from matings expected to yield 50 percent heterozygotes than from matings producing only homozygotes. This consistent association of parental genotypes with traits being measured in different phases of the "juvenile" period suggests that favorable allele effects may alternate within a fairly short time span (for example, within the incubation period) or that the two alleles in the heterozygote may simultaneously perform complementary physiological functions affecting two or more biologically distinct phases of the life cycle.

In addition to the effects on livability and egg production described in this study and in earlier reports (SHULTZ and BRILES 1953; BRILES 1954, 1956; KRUEGER, BRILES and WOODS 1956), hatchability (BRILES and KRUEGER 1955), and growth rate (BRILES, JOHNSON and GARBER 1953; C. BRILES 1956; BRILES 1957) have also been shown to be associated with the *B* system genotypes. In

accounting for these associated effects, one might hypothesize that they are due entirely to closely linked genes. If the observed associations were *solely* due to linked genes, crossing over under conditions of equilibrium should result in equal frequency of coupling and repulsion linkages with the various *B* alleles present in the population. At this point the associated effects would no longer be observed on the population level, and the *B* locus would be free to become homozygous without deleterious effects. Neither the data from the present study nor that from the work cited above have involved a sufficient number of generations to give any meaningful information regarding the probable role of linked genes. On the other hand, there is evidence that the *B* locus itself plays a significant role in accounting for at least some of these effects—especially those related directly to reproductive fitness. First, multiple allelism at the *B* locus is universally characteristic of relatively noninbred populations (BRILES, MCGIBBON and IRWIN 1950; SHULTZ and BRILES 1953; BRILES *et al.* 1957; KIMMELL 1957; FANGUY 1958). Second, inbreeding is relatively ineffective in fixing alleles at the *B* locus (BRILES and MCGIBBON 1948; BRILES 1949; SHULTZ and BRILES 1953; BRILES *et al.* 1957; GILMOUR 1954, 1958, 1959). These two findings indicate strongly that heterozygosity at the *B* locus is involved in fitness on the population level. The association of the *B* genotypes with quantitative physiological traits (livability, egg production, etc.) most likely represent specific manifestations of this locus that are measurable on an individual level; the optimum integration of such effects over the life span of individuals of the population could easily account for the multiple allelism characteristic of this locus in noninbred populations and for the persistence of two or more alleles under inbreeding.

A third possibility, especially attractive in accounting for the magnitude of the effects, is that the *B* blood group locus may be a heterotic locus around which have accumulated interacting loci under the supergene concept (FISHER 1930; MATHER 1943, 1955; SHEPPARD 1953, 1955). Assuming the establishment of a polymorphic locus, the development of a supergene is based on the expected increase in linkage between such a locus and others with which it interacts. The increase in linkage results from natural selection favoring chromosomal rearrangements which reduce the distance between the loci concerned. The essential effect of this tighter linkage is to increase the frequency of simultaneous segregation of genes having favorable "combination effects". It is evident from present serological and genetic data that such closely linked loci would likely be involved only in accounting for some of the physiological end effects; the serological complexity observed at the *B* locus is amply accounted for by multiple alleles, each responsible for the specificity of a unitary, yet chemically complex, agglutinogen.

The data presented here for the *B* blood group system lends general support to the genetic homeostasis concept as advanced by LERNER (1954). Furthermore, it appears that the accumulative or total "life-span" effect resulting from alternate superiority of alleles may play a role in accounting for the over-all superiority of the heterozygote over both homozygotes. Seemingly contradictory to the general thesis that heterozygotes are more effectively buffered against environ-

mental fluctuations than homozygotes is the finding that the heterozygotes in the various lines interacted strongly with yearly environmental differences for both juvenile and adult livability (Table 6); whereas among the homozygotes interaction with years occurred only with those displaying the lower livability values in the two periods (homozygote 2 in the juvenile and homozygote 1 in the adult). In other words, the homozygous classes which appeared to exert the more favorable influence in the respective livability periods (homozygote 1 in the juvenile and homozygote 2 in the adult) did not exhibit interaction with years, showing that these homozygous classes over the several lines responded in similar fashion to annual shifts in environmental conditions.

These results suggest that inbreeding may have resulted in the disruption of normal genic balance (where the heterozygote is assumed to be better buffered). A normally high level of physiological activity associated with heterozygotes under a balanced total genotype could become highly sensitive with a loss of buffering when superimposed on the relatively homozygous backgrounds afforded by the various inbred lines. Residual heterozygosity at particular loci, as represented here by the *B* locus, on otherwise highly homozygous backgrounds could play a role in accounting for the relatively large amounts of variance in inbreds compared to their hybrids (see review by LERNER 1954). On the other hand, there is a possibility that the individuality of the particular heterozygotes as disclosed by their genotype × year interaction may be characteristic of *B* heterozygotes in normal panmictic populations. The selective advantage on a *population level* afforded by supplementary action of the environmental adaptiveness of the various heterozygotes could play a role in accounting for the extensive multiple allelism characteristic of the *B* locus.

## SUMMARY

Heterozygous and homozygous genotypes at the *B* blood group locus were compared with respect to their effects on livability from fertilization to 150 days of age (juvenile period), on livability during the period from 150 days to 450 days of age (adult period), and on the rate of egg production. Six inbred lines having two *B* alleles each and a seventh line possessing three *B* alleles supplied nine sets of full-sib heterozygous-homozygous comparison groups. Over a three-year period, a total of 7,732 fertile eggs from 279 matings between parents of known blood type supplied families in which the number of homozygotes and heterozygotes surviving were compared to that expected on the basis of random segregation and fertilization. For each line paired comparisons of the three genotypic classes resulting from each set of two alleles were made within families for both juvenile and adult livability. The data from chicks surviving the juvenile period showed that livability differences were associated with the *B* genotypes in two of the lines—H2 and H8. Differences in adult livability were also found to be associated with *B* genotypes in two lines—H1 and H2.

In an effort to facilitate the disclosure of *B* genotype effects which might apply to several or all of the lines, the homozygotes in each of the two-allele comparison groups were ranked on the basis of their relative favorable effect on juvenile liva-

bility into types 1 and 2, respectively. Pooling each of these types over the seven lines revealed that there was interaction between homozygotes of types 1 and 2 in the juvenile and adult livability periods, indicating that at least in certain lines there was a shift in relative effectiveness of the two homozygous types as the animal passed from one phase of its development to the next.

Interaction between genotypes and years (annual environments) within the juvenile and adult livability periods was revealed for the heterozygotes in both periods, for homozygotes of type 1 in the adult period, and for homozygotes of type 2 in the juvenile period. Interaction with years did not occur for homozygotes of type 1 in the juvenile period or for homozygotes of type 2 in the adult period. Some implications of these findings are dicsussed.

Variation in rate of egg production was affected by the *B* blood group genotypes in three of the lines. In general, the effect resulted from one of the homozygous types laying at a lower rate.

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# GALACTOSE MUTANTS OF SALMONELLA TYPHIMURIUM

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IT is now well established that the fermentation of galactose by many organisms involves the sequential action of three specific enzymes: galactokinase (kinase), Gal-1-P uridyl transferase (transferase), and UDPGal-4-epimerase (epimerase) (KALCKAR 1958). (Abbreviations: Gal-1-P, $\alpha$-galactose-1-phosphate; UDPGal, uridine diphosphogalactose.) Recently, KALCKAR, KURAHASHI and their co-workers (KALCKAR, KURAHASHI and JORDAN 1959; KURAHASHI 1957) have carried out biochemical studies on many galactose-negative mutants of *Escherichia coli* strain K-12 which had been isolated and genetically analyzed by the LEDERBERGS and associates (MORSE, LEDERBERG and LEDERBERG 1956a,b; LEDERBERG 1960). In the course of these investigations, it was discovered that the mutants lacking transferase induce themselves into a state called "galactose-induced bacteriostasis" (YARMOLINSKY, WIESMEYER, KALCKAR and JORDAN 1959), when they are grown in the presence of galactose.

On the other hand, another type of galactose-sensitive mutants (M mutants) was described by MURASE of our laboratory in 1932 (MURASE 1932), and was studied in detail by us (FUKASAWA and NIKAIDO 1959b; NIKAIDO 1961). These mutants were found to have a single defect in the enzyme epimerase, and to show remarkable "galactose-induced bacteriolysis" when grown in the presence of galactose (FUKASAWA and NIKAIDO 1959a, 1961).

This report is mainly to describe the results of transductional analysis of *Gal* loci using M mutants and other galactose nonfermenters of *Salmonella typhimurium*. In addition, the results of enzymatic analysis and growth studies on these mutants will be described and finally, genetic control of galactose metabolism will be discussed on the basis of these results. [A part of this study was reported at the 32nd general meeting of the Genetics Society of Japan (1960). A preliminary report has also appeared (NIKAIDO and FUKASAWA 1961).]

## MATERIALS AND METHODS

*Bacterial strains:* (1) *Salmonella typhimurium* strain LT-2 and its auxotrophic derivatives, *tryD-10*, *leuB-28*, *pro-13*, originally isolated in DR. M. DEMEREC's laboratory. (2) Eighteen M mutants which were independently isolated from both LT-2 and its auxotrophic derivatives. These M mutants were isolated according to the original method of MURASE: Twenty tubes containing 5 ml nutrient broth were inoculated with LT-2 or its derivatives and were incubated at 37°C

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for two weeks. A loopful of each culture was spread every day from each tube onto modified Endo agar plates containing galactose instead of lactose. M mutants can be recognized by their nonfermentation of galactose and by their peculiar colony morphology; they give rise to very flat colonies, with somewhat concave centers on agar plates containing galactose. (3) Five galactose nonfermenting mutants originated from *S. typhimurium* strain LT-7; *gal-5*, *-9*, *-13*, *-21* and *-24*, which were kindly supplied through Dr. T. Miyake by Drs. M. Demerec and Z. Hartman. These strains were reported by them to belong to the same transductional group (Z. Hartman 1956; Demerec *et al.* 1954). (4) *S. typhimurium* strain LT-7 and an M mutant derived from it—*7M1*.

*Phage:* P22 was used (Zinder and Lederberg 1952).

*Media:* BTB-galactose medium (nutrient agar containing bromthymol blue and 2% D-galactose (Difco), was used for selecting galactose-positive transductants. Nutrient broth, penassay broth (Difco), and tryptone broth (1% tryptone (Difco), 0.5% NaCl) were used as liquid media.

*Transduction experiment:* One tenth ml of 18 hour culture of recipient strains in penassay broth was plated on BTB-galactose plate with 0.1 ml of phage stock grown on donor strains (Morse *et al.* 1956a). After 48–72 hour incubation at 37°C, transductant colonies developed as yellow papillae on the heavy, blue background. Numbers of transductants were shown to be proportional to the amount of input phage in our experimental conditions. Phage was prepared by two cycles of growth on log-phase, penassay broth cultures of the donor strains and freed from bacteria by low speed centrifugation before sterilization with chloroform. For growing the phage on M mutants, D-galactose was added to the broth in the concentration of 0.1 percent, because M mutants of Salmonella are resistant to P22 and their sensitivity to the phage is recovered during their growth in the presence of galactose (Fukasawa and Nikaido 1960; Fukasawa 1961). Approximate titer of transducing phage was usually determined as the plaque forming units on wild-type strain. Phage P22 was shown, in preliminary experiment, to be able to form nearly the same numbers of plaques on M cells as on wild-type cells under the presence of 0.1 percent of galactose and 1.0 percent glucose, where glucose was added for preventing galactose-induced lysis of M cells.

*Preparation of extracts for the assay of enzymes:* Cells grown overnight in nutrient broth were centrifuged and resuspended in 150–200 ml fresh broth and were agitated in a water bath at 37°C. During their exponential phase of growth, D-galactose (0.1 percent in the final concentration) was added. After 30 min, the suspension was rapidly chilled in ice bath, centrifuged in the cold, washed twice with ice-cold water, and the cells ground with alumina for 20 min in a mortar chilled in ice. The paste was extracted with distilled water and the extract centrifuged for 15 min at 10,000 rpm. (Noninduced extracts were prepared in the same way without addition of galactose.) The clear supernatant was used for the assay.

*Assay of the enzymes on the Leloir pathway:* Enzyme assay was performed as described by Nikaido (1961).

*Test of sensitivity to galactose:* Tryptone broth was used for this experiment. All cultures were grown in 200 ml Erlenmeyer flasks with shaking in a water bath at 37°C and D-galactose (recrystallized twice from D-galactose (Difco)) was added to 0.1 percent at the exponential phase of their growth. Growth was followed by optical density readings at 660 mμ.

*Sensitivity to galactose of the strains used:* As is seen in Figure 1B, all M mutants tested underwent severe lysis at 30 min after galactose was added. Out of five galactose nonfermenters of Z. HARTMAN, four strains, *gal-9*, *-13*, *-21* and *-24*, showed neither lysis nor stasis under our conditions (Figure 1A). Strain *gal-5* exhibited a particular type of sensitivity to the sugar; after addition of galactose, growth of this strain proceeded at a reduced rate for 30–120 min and then slight lysis seemed to occur (Figure 1C).

## RESULTS

*Activities of the enzymes in the strains used:* The results are shown in Table 1. M mutants were confirmed to have a defect in UDPGal-4-epimerase. Four

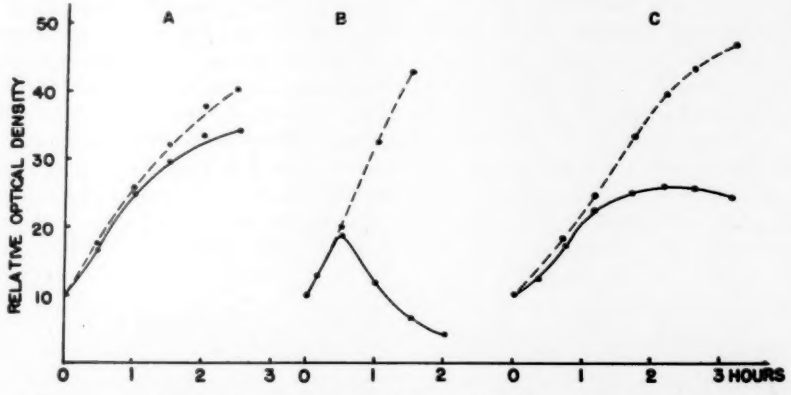


FIGURE 1.—Cells were grown in tryptone broth and at their exponential phase of growth galactose was added to 0.1 percent. The time of addition is shown as time 0. Dotted lines show controls without galactose. A: *gal-24*, B: *7M1*, C: *gal-5*.

TABLE 1

*Induced levels of the enzymes on the Leloir pathway in* S. typhimurium *strain LT-2, LT-7 and the various galactose-negative mutants derived therefrom*

| Strains | Specific activity (micromoles/mg protein/hr) | | |
|---|---|---|---|
| | Galactokinase | Gal-1-P uridyl transferase | UDPGal-4-epimerase |
| LT-2, LT-7 | 5.2–5.5 | 9.9–25.6 | 54–67 |
| *gal-9*, *-13*, *-21*, *-24* | <0.2 | 10.1–13.4 | 29–50 |
| *M-1*, *-3*, *-5*, *-6*, *-10*, *-18* | 2.5–4.5 | 7.3–13.2 | <0.003 |
| *gal-5* | <0.2 | 0.17 | 0.05 |

strains, *gal-9*, *-13*, *-21* and *-24*, were found to have a single defect in galactokinase. Strain *gal-5* was found to have triple defects in these three enzymes, but the loss of Gal-1-P transferase and UDPGal-4-epimerase was not complete, and traces of kinase might be present, although our method of assay is not sensitive enough to give definite results.

*Constitutivity of the enzymes in various mutants:* Extracts were prepared also from noninduced cells grown in broth without addition of galactose. Enzyme activities were determined on these extracts and were compared with those of induced cells (Table 2). In most experiments, induction period was limited to 30 minutes because M cells showed extensive bacteriolysis after this period. But since it was demonstrated that the full development of these enzymes required about 150 minute induction with galactose in wild-type cells, experiments were carried out with some strains using this longer induction period, and in Table 2, these results are shown in parentheses.

As is evident from the table, in all four kinaseless mutants the i/n ratios are markedly decreased than the wild type. Thus, the mutations in kinase gene exerted a pleiotropic effect to make the synthesis of transferase and epimerase constitutive. In a galactose-positive revertant from *gal-9*, the synthesis of these enzymes was inducible, and the synthesis of kinase and transferase did not seem to be affected by mutations in the epimerase gene, since an M mutant derived from LT-*7* (*7M1*) and *M-1*, *-3*, *-5*, *-6*, *-10* and *-18* derived from LT-2 exhibited typically inducible synthesis of these enzymes.

*Reciprocal transductions between M mutants:* When two M strains out of 18 available in our collection were used for transduction as a donor and a recipient respectively, a small number of galactose-fermenting transductants were produced in some combinations, whereas no transductants could be found in other combinations. As a result of these experiments, the 18 M mutants were classified

TABLE 2

*Constitutivity of the enzymes on the Leloir pathway in* S. typhimurium *strain LT-7 and mutants devived therefrom*

| | | Specific activity (micromoles/mg protein/hr) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Galactokinase | | | Gal-1-P uridyl transferase | | | UDPGal-4-epimerase | | |
| Strain | Genotype | non-induced | induced | i/n | non-induced | induced | i/n | non-induced | induced | i/n |
| LT-*7* | *k*$^+$*t*$^+$*e*$^+$ | 0.5 | 3.7 | 7.4 | 0.78 | 12.6 | 16.2 | 4.3 | 62 | 14.4 |
| | | | | | | (26.0) | (33.4) | | (141) | (32.8) |
| *gal-9* | | .. | .. | .. | 10.4 | 10.1 | 1.0 | 46 | 31 | 0.7 |
| | | | | | | (21.6) | (2.1) | | (39) | (0.9) |
| *gal-13* | *k*$^-$*t*$^+$*e*$^+$ | .. | .. | .. | 4.8 | 13.4 | 2.8 | 36 | 43 | 1.2 |
| | | | | | | (16.3) | (3.4) | | (36) | (1.0) |
| *gal-21* | | .. | .. | .. | 5.1 | 11.2 | 2.2 | 46 | 50 | 1.1 |
| *gal-24* | | .. | .. | .. | 3.0 | 10.2 | 3.4 | 18 | 29 | 1.6 |
| *gal-9R* | *k*$^+$*t*$^+$*e*$^+$ | 0.3 | 4.4 | 14 | 1.7 | 15.1 | 8.9 | 14 | 70 | 5.0 |
| *7M1* | *k*$^+$*t*$^+$*e*$^-$ | 0.3 | 2.5 | 8.3 | 0.50 | 5.3 | 10.6 | .. | .. | .. |

Figures in parentheses show values obtained on extracts of the cells induced for 150 min in the presence of 0.2 percent galactose. i/n denotes the ratio specific activity of the extract of induced cells/specific activity of the extract of noninduced cells.

into six groups: group (a) *M-1*, *-2*, *-4*, *-7*, *-8*, *-9*, *-11*, *-12*, *-14*, *-15*, *-16*. (b) *M-3*, *-13*. (c) *M-5*. (d) *M-6*. (e) *M-10* and (f) *M-18*.

*Reciprocal transductions between the galactose-negative mutants:* Six M mutants which are the representatives of six groups, *M-1*, *-3*, *-5*, *-6*, *-10* and *-18*, and five other galactose negative mutants, *gal-5*, *-9*, *-13*, *-21* and *-24*, were used in this experiment (Table 3). When two M strains were used as a donor and a recipient respectively, small numbers of galactose-fermenting transductants developed, whereas when the wild type or any other galactose-nonfermenter was used as a donor and M mutants as recipients, larger numbers of galactose-fermenters were produced. Accordingly, it can be concluded that the M mutants which are defective in epimerase, belong to one transductional group. In the same way, the five strains of Z. HARTMAN, four kinaseless and one triply defective, were confirmed to belong to another transductional group, as reported by her (1956). But it should be mentioned here that triply defective strain *gal-5* mapped somewhat apart from the other four kinaseless strains. Thus, the results of transductional analysis are in perfect agreement with the classification of the mutants by their phenotypes. Moreover, it was demonstrated that linkage existed between these two transductional groups; when the mutants of epimeraseless group were infected with phage from the strains of kinaseless group, the numbers of galactose-positive transductants were less than those obtained by the infection with phage from the wild type. The same relationship was observed also in the reciprocal cross of the opposite direction. In these experiments, *gal-5* was not used as a donor since phage did not grow well on this strain.

## DISCUSSION

Although the genetic analysis of *Gal* loci has been extensively carried out in

TABLE 3

*Transductions between galactose nonfermenters of* S. typhimurium

| Recipients | Donors | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *gal-9* | *-13* | *-21* | *-24* | *M-1* | *-3* | *-5* | *-6* | *-10* | *-18* | + |
| *gal-9* | 0 | 0 | 0 | 0 | 145 | 152 | 290 | 890 | 495 | 141 | 4100 |
| *gal-13* | 2 | 0 | 14 | 5 | 563 | 1000 | 700 | 218 | 340 | 352 | 5450 |
| *gal-21* | 40 | 5 | 0 | 37 | 882 | 1100 | 152 | 348 | 500 | 520 | 3900 |
| *gal-24* | 18 | 23 | 8 | 0 | 198 | 135 | 320 | 442 | 880 | 720 | 6300 |
| *gal-5* | 49 | 83 | 27 | 33 | 230 | 112 | 60 | 107 | 110 | 118 | 9400 |
| *M-1* | 720 | 890 | 220 | 345 | 0 | 73 | 9 | 28 | 43 | 21 | 5280 |
| *M-3* | 375 | 345 | 163 | 145 | 27 | 0 | 1 | 32 | 43 | 46 | 3370 |
| *M-5* | 214 | 740 | 198 | 400 | 94 | 45 | 0 | 189 | 18 | 163 | 10580 |
| *M-6* | 530 | 770 | 240 | 267 | 153 | 395 | 36 | 0 | 21 | 18 | 6430 |
| *M-10* | 740 | 650 | 155 | 240 | 145 | 353 | 22 | 64 | 0 | 71 | 4590 |
| *M-18* | 440 | 445 | 128 | 170 | 41 | 113 | 24 | 19 | 54 | 0 | 9660 |

Figures in the table denote the total numbers of transductants selected on two BTB-galactose agar plates when about $10^8$ bacteria and $10^9$ plaque forming units of transducing phage were plated on each plate (in the experiments with phage from wild type, $10^8$ plaque forming units were plated). Numbers of transductants were calculated by subtracting the total numbers of spontaneous revertants on two phage-free plates from those of galactose fermenting colonies on two transduction plates. Phage titre was determined on the wild type strain.

*Escherichia coli* strain K-12 by the LEDERBERGS and associates and the phenotypic analysis by KALCKAR, KURAHASHI, and others, mutants singly defective in epimerase have not yet been reported by them. It had once been discussed whether the mutation to the lack of epimerase might be lethal because of the failure of synthesis of galactose (KALCKAR 1958). This type of mutant, however, had already been isolated as early as 1932 from several genera of Enterobacteriaceae by MURASE of our laboratory. But the nature of biochemical defect in these mutants remained almost unknown until we have described in detail the phenotypic characteristics in previous papers (FUKASAWA and NIKAIDO 1959a,b, 1961; NIKAIDO 1961).

The mechanism of selective advantage of M mutants in aged broth cultures is yet unknown, but it is assumed that they have point mutations at one locus—epimerase gene, for the following reasons. (1) All the M mutants tested can give rise to wild-type transductants when infected with phage grown on wild-type, kinaseless mutants, and appropriate M mutants respectively, (2) they revert to wild type at a frequency ranging from $10^{-7}$ to $10^{-8}$, and finally, (3) by transductional analysis all of them were shown to belong to the same transductional group.

As already described, M mutants have several characteristics in addition to the inability to ferment galactose. (1) They are sensitive to galactose and show galactose-induced bacteriolysis, (2) they form somewhat rough colonies on ordinary media, (3) they are resistant to phage P22, and (4) they have cell walls of a peculiar sugar composition containing only glucose. Phenotypically wild-type revertants and transductants obtained from M mutants were examined on these points and were found to have reverted to wild-type characteristics in all these respects: They were resistant to galactose, formed smooth colonies, were sensitive to P22, and contained galactose, mannose, rhamnose, and 3,6-didesoxyhexose in their cell wall in addition to glucose. These pleiotropic effects of a single mutation might be, as already shown (FUKASAWA and NIKAIDO 1961), explained biochemically as necessary consequences of a single defect in epimerase.

The presence of the so-called "complex loci" has been demonstrated in a number of genes, especially in Salmonella (DEMEREC and HARTMAN 1956; DEMEREC and HARTMAN 1959). Our analysis of 18 M mutants by reciprocal transduction with P22 has also revealed at least six nonidentical mutational sites in epimerase locus.

Through transductional crosses between wild-type, kinaseless mutants and epimeraseless (M) mutants, the presence of close linkage was demonstrated between kinase and epimerase genes, although their precise mapping was not attempted, because other linked markers are not known for doing the three-point test. It has been shown that, in this organism, the genes controlling a sequence of metabolic reactions, i.e., tryptophan synthesis, histidine synthesis, and so on, are arranged close together (DEMEREC and Z. HARTMAN 1956; P. HARTMAN 1956), a fact, which among others has led JACOB, PERRIN, SANCHEZ and MONOD (1960) to the formulation of the *operon* concept. In view of our findings reported here, it seems most probable that the genes controlling the three enzymes of

galactose metabolism are located close together on the chromosome, although transferaseless mutants were not available and the localization of transferase gene is therefore obscure.

Strain *gal-5* seems to have been derived by the mutation $o^o$ in operator gene but not in classical structural genes for the following reasons[1]: (1) The strain was isolated apparently by a single mutational event; (2) It reverts to galactose fermenter at the frequency of $10^{-7}$ to $10^{-8}$; (3) Transduction of galactose fermentation occurs when wild type or any other galactose nonfermenter is used as a donor. (Results similar to these were already reported by Z. HARTMAN. These findings indicate that *gal-5* originated from point mutation.); (4) The mutated gene locus of the strain is found to be linked to those of other galactose mutants tested; (5) This mutant is defective simultaneously in kinase, transferase and epimerase. (The same class of mutation was reported in *E. coli*, by LEDERBERG (1960) and KALCKAR *et al.* (1959), and JACOB *et al.* (1960) pointed out that it might be due to the mutation $o^o$ in operator gene.) These findings, together with the finding that structural genes controlling the enzymes of galactose metabolism are closely linked, suggest that these genes are probably organized to form an *operon* on the chromosome. It was already reported by KALCKAR *et al.* (1959) that in two kinaseless mutants of *E. coli* strain K-12, transferase alone, and not epimerase, is synthesized constitutively. We have found that in four kinaseless mutants of *S. typhimurium* the synthesis of transferase and also epimerase is constitutive, despite the fact that their synthesis is typically inducible in wild-type cells and in a galactose-positive revertant from one of these mutants. [We were recently informed that DR. H. M. KALCKAR has independently discovered the constitutive synthesis of epimerase in kinaseless mutants of *E. coli*. (Personal communication to DR. K. KURAHASHI).] Thus, point mutations which occurred in a specific structural gene, kinase gene, seem to have a pleiotropic effect of making the synthesis of other two enzymes controlled by the *operon* more or less constitutive. This *coordinate, simultaneous* "derepression" of the synthesis of two enzymes is in perfect accord with the *operon* concept. In general, the constitutive synthesis of normally inducible enzyme is known to occur as a result of either mutation $i^-$ in the regulatory gene or mutation $o^c$ in the operator gene. In the present case, no experimental finding has been obtained on the mechanism of the constitutivity. It might, however, be suggested by the very close linkage between kinase and operator genes in this system, that the mutations in the kinase gene have effects simulating operator mutation $o^c$. But on the other hand, it was reported that in kinaseless mutants of *E. coli* K-12, the constitutivity was recessive to the inducibility in reciprocal heterogenotes with *lambda-gal* (YARMOLINSKY and WIESMEYER 1960), a result suggesting that mutations in the kinase gene exert their effect through a cytoplasmic factor.

It is interesting that kinaseless mutants have been found in nature which do not show constitutive synthesis of transferase or epimerase. For example, a

[1] It should be mentioned here that noncomplementability with other classes of galactose mutants, one of the important features of $o^o$ mutation, was not yet established because of the technical difficulties in carrying out the complementation test with *gal-5*.

mutant of yeast studied by ROBICHON-SZULMAJSTER (1958) had typically inducible transferase and epimerase. In addition, it might be worth noting that a kinaseless mutant (W-3142) of *E. coli* K-12 seemed to be weakly inducible in transferase synthesis (KALCKAR *et al.* 1959), and that in the mutant, the locus of mutation seems to be located somewhat apart from the galactose gene cluster (LEDERBERG 1960).

In order to clarify further the genetic control of the enzyme formation in the present system, it should be required not only to determine the order of genes within *galactose operon* more precisely through a three-point test but also to apply cistron analysis to it by some means, for example by abortive transduction analysis. These experiments will be conducted in future.

## SUMMARY

Three types of galactose nonfermenting mutants of *Salmonella typhimurium* strains LT-2 and LT-7 were described and utilized for the genetic analysis of *Gal* loci in this organism. These were: (1) Epimeraseless mutants, which have been called M mutants in our laboratory and which have not been found so far in *Escherichia coli* strain K-12. (2) A tripleless mutant, which was defective in kinase, transferase, and epimerase, and seemed to be due to a mutation $o^o$ in operator gene. (3) Kinaseless mutants, which were not only defective in kinase, but also capable of constitutive formation of the other two enzymes involved in galactose metabolism.

Through transductional analysis, it was demonstrated that epimeraseless mutants belong to one transductional group and that tripleless and kinaseless mutants to another group. These two groups were linked closely enough to be recognized by the transduction with P22. From these results, it was assumed that the genes of *Gal* loci in *Salmonella typhimurium* are organized to form an integral unit: *operon*. In addition, 18 epimeraseless mutants were classified into six subgroups, indicating that epimerase locus has at least six nonidentical mutational sites.

On the basis of these findings, genetic control of galactose metabolism is discussed.

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# TRIPARENTAL MATINGS IN ESCHERICHIA COLI

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THE present work was carried out with the purpose of establishing whether a single female cell ($F^-$) of *E. coli* K-12 could mate with two male cells (Hfr) at the same time, leading to a triploid merozygote. The degree of triploidy postulated would depend, in fact, on the type of males used, that is, on the common portion of the particular group of markers transmitted by each of them (JACOB and WOLLMAN 1957).

LEDERBERG (1947) investigated the possibility of triparental crosses in *E. coli* K-12, but at that time mating types had not yet been recognized in this organism (LEDERBERG, CAVALLI-SFORZA and LEDERBERG 1952; HAYES 1953; CAVALLI-SFORZA, LEDERBERG and LEDERBERG 1953), nor conjugation features established (WOLLMAN and JACOB 1955; LEDERBERG 1957). The experiment tested a *ménage à trois* of two females with one male, and present knowledge would lead one to expect a negative outcome, as was actually observed.

Regarding other microorganisms, triparental recombination has been recently discovered in yeasts by POLSINELLI (1960).

Occurrence of triploid or partially triploid zygotes by mechanisms other than a triparental cross is known in a variety of organisms. We are not going to summarize them here, as it is not our purpose to discuss patterns of segregation.

In connection with the problem investigated, was the question of whether a fertilized $F^-$ acquires some kind of immunity against further matings. Some of the experiments described in this paper may furnish an indirect answer also to this latter question.

## MATERIALS AND METHODS

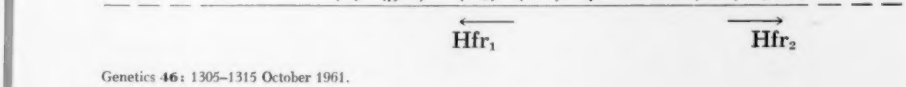
*Strains:*

439: $F^-$ *leu pro str*
682: $Hfr_1$ *leu* + +
683: $Hfr_1$ + *pro* +
U 187: $Hfr_2$ + *pro* +
684: $Hfr_2$ *leu* + +

only markers with a bearing on the experiments described in this paper have been cited.

(Numbers refer to L. L. CAVALLI-SFORZA's list of stocks, except for U 187, kindly supplied by E. CALEF, a lysogenic derivative of a strain received from E. LEDERBERG as W 3980.)

Position of markers on the linkage map of *E. coli* K-12 (imagining a portion of the circular map (JACOB and WOLLMAN 1957) stretched out):



Pedigrees of strains:

682: recombinant from a cross 622 ($Hfr_1$ *met*) × 439 ($F^-$ *leu pro gal*) selected on EM† *gal leu.*

683: recombinant from a cross 622 × 439 selected on EM *gal pro.*

684: recombinant from a cross U 187 × 392 ($F^-$ *his leu*‡) selected on MM *leu.*

† Minimal eosine-methylene blue medium without any other carbon source than the sugar added as required (LEDERBERG).

‡ Same *leu* mutation as 439′.

*Media:* Difco Penassay, as broth or agar, was used as a complete growth medium. DAVIS' formula (DAVIS and MINGIOLI 1950) was followed for minimal medium (MM), supplemented when required.

*Crossing procedure:* Both female and males were grown in broth to logarithmic phase in a rotator, centrifuged, and resuspended in fresh Penassay broth. Crosses were performed with gentle shaking in a water bath at 37°C with a volume varying from 1.5 ml to 4.5 ml in 100 ml Erlenmeyer flasks to give efficient aeration. For interruption of conjugation, vibration by a rotating eccentric rubber wheel was employed. Tenfold dilutions were made at given times in 1.8 ml of cold saline, whether interrupted or not, further diluted if necessary, and kept at ice temperature till plating was performed.

Plating on selective minimal media was done inoculating a top layer of 4 ml of MM without any supplementation (diffusion from the bottom layer was sufficient) in order to decrease plate recombination. Plates, and tubes containing 4 ml of melted agar, were previously kept at 40°C–45°C. Titration on complete medium was performed in a top layer of soft agar. Incubation was at 37°C.

## EXPERIMENTAL RESULTS

*Occurrence of triparental recombinants:* A mating mixture (*ménage à trois*) was prepared as follows: a streptomycin resistant $F^-$, having a double requirement for leucine and proline (439), plus a streptomycin sensitive, leucine requiring $Hfr_1$ (682), plus a streptomycin sensitive, proline requiring $Hfr_2$ (U 187). Four possible types of crosses could take place in such a mixture. Of these, the first three are known to occur, the fourth one was under investigation:

(1) 439 × 682: recombinants from this cross could be selected on MM *str leu.*

(2) 439 × U 187: recombinants from such a cross could be selected by plating on MM *str pro.*

(3) 682 × U 187: this type of progeny could be selected on MM.

(4) 439 × 682 × U 187: plating on MM *str*, the only cells able to grow should necessarily have received a contribution from each of the three parents.

Numbers of colonies grown on these different media from the same mating mixture described above are given in Table 1.

*leu* and *pro* markers were chosen since they are introduced at high frequency by both of the two males used (JACOB and WOLLMAN 1957). This was confirmed

TABLE 1

*Frequencies of recombinants from the four possible types of crosses occurring in a* "ménage à trois"

| Mating mixture viable cells/ml | 439: $1.2 \times 10^8$<br>682: $2.2 \times 10^8$<br>U 187: $0.82 \times 10^8$ | F⁻<br>$Hfr_1$ *leu*<br>$Hfr_2$ *pro* |
|---|---|---|
| Selective medium and origin of recombinants | Time of contact (minutes) 0 | 30 |
| MM *leu str* (439 × 682) | $<10^2$ | $2.8 \times 10^6$ |
| MM *pro str* (439 × U 187) | $<10^2$ | $1.1 \times 10^6$ |
| MM (682 × U 187) | $10^{4*}$ | $7.3 \times 10^6$ |
| MM *str* (triparental) | $<10^2$ | $1.1 \times 10^4$ |

Time 0 minutes: interrupted immediately after mixing on a tenfold dilution, of which 0.1 ml were plated.

Time 30 minutes: diluted without interruption. Inocula of 0.1 ml of a $10^{-3}$ dilution, except for MM *str*, where 0.1 ml of a $10^{-1}$ dilution were plated.

Controls of spontaneous reversion for each marker and for each strain: $<0.3 \times 10^2$.

* Platings on MM always gave some plate recombination, probably due to residual growth.

by platings on MM *str leu* and MM *str pro* (Table 1), showing for types (1) and (2) crosses a recombination frequency of about one percent which was the usual value, in our experimental conditions, for a normal F⁻ × Hfr cross.

From the platings on MM, it may be noted that the recombination frequency of the Hfr × Hfr cross was found to be unexpectedly high. This will be discussed later.

As for colonies grown on MM *str* (Table 1), which appear at a much lower frequency (about $10^{-4}$ of the original mixture), they must have arisen, as said before, through a contribution from all three parents.

The possibility of recombination between nonallelic markers of only one of the two males and the female was excluded, for *leu* marker by pedigree and for *pro* by allelism tests.

Having thus established the occurrence of triparental recombinants, the following questions were formulated:

(a) Is a triparental cross conditioned by the presence of different Hfr's (in our case $Hfr_1$ and $Hfr_2$) or is it possible also with males of the same type? [Note—$Hfr_2$: Strain U 187, actually, as shown by subsequent experiments of interrupted mating, seemed to have an abnormal $Hfr_2$ type, introducing *leu* only at about 40 minutes (but still retaining a high frequency for *gal* and *lambda*).]

(b) What events lead to the triparental mating? A first trivial possibility, namely mating of the second male with a segregant from a cross of the female with the first male, is ruled out by the structure of the experiment. In fact, triparental recombinants are already present in the mixture after 30 minutes of contact (and earlier; see Table 3). We know from data of WOLLMAN, JACOB and

HAYES (1956) and of TOMIZAWA (1960) that segregation does not take place for at least 80 minutes after time of mixing. So we are left with two other possibilities:

(1) three cells (two male and one female) can mate simultaneously, forming a triplet (one-stage process).

(2) A female mates with one male; after transfer of the male material, the fertilized female (merozygote) can receive additional genetic material from other male cells (two-stage process).

These two hypotheses are not necessarily mutually exclusive. In both cases, formation of a partially triploid zygote is postulated.

*Absence of an effect of Hfr type:* To answer the first question, the same female (439) was mixed in turn with the four compatible pairwise assortments of four different Hfr's—two $Hfr_1$ and two $Hfr_2$. Results are summarized in Table 2. It can be seen that a triparental cross is established independently of whether or not the Hfr's are of the same type, as recombinants on MM *str* occur in the four mixtures at about the same frequency.

*Kinetics of triparental crosses:* Concerning the main question, whether triploid zygotes were formed by a one-stage or a two-stage process, the kinetics of appearance of triparental recombinants, with regard both to entry of markers and to conjugation itself, could supply some critical information. A combined experiment of interrupted and noninterrupted mating was performed with the same mating mixture. Controls were run as usual of the two "legitimate" types of crosses (Hfr × F⁻) occurring in the mixture. These, for interrupted samples, gave the actual time of entry, for the given experiment, of each one of the two markers considered.

In Figure 1 are plotted numbers of colonies grown on the different selective media from the interrupted samples. The appearance of recombinants is at about ten minutes for *str*$^r$ *pro*$^+$ progeny (from 439 × 682 crosses = C 1), and at about 20 minutes for *str*$^r$ *leu*$^+$ progeny (from 439 × 683 crosses = C 2); according to the sequence expected from $Hfr_1$.

*str*$^r$ *pro*$^+$ *leu*$^+$ progeny (from triparental crosses = M) follow closely the kinetics of the later marker, beginning to appear as soon as this one enters.

TABLE 2

*Effect of Hfr types on the occurrence of triparental recombinants*

| Selective medium | Viable cells/ml | Mating mixtures (I) 439:1.6×10⁸ 682:1.6×10⁸ U 187:1.9×10⁸ | (II) 439:1.6×10⁸ 684:3.3×10⁸ 683:1.7×10⁸ | (III) 439:1.6×10⁸ 682:1.6×10⁸ 683:1.7×10⁸ | (IV) (439:1.6×10⁸ 684:3.3×10⁸ U 187:1.9×10⁸ | F⁻ Hfr *leu* Hfr *pro* |
|---|---|---|---|---|---|---|
| MM *str* | | $1.96 \times 10^4$ | $1.8 \times 10^4$ | $2.05 \times 10^4$ | $0.63 \times 10^4$ | |
| MM *leu str* | | $1.45 \times 10^6$ | $2.15 \times 10^6$ | $1.96 \times 10^6$ | $1.74 \times 10^6$ | |
| MM *pro str* | | $0.88 \times 10^6$ | $2.0 \times 10^6$ | $0.98 \times 10^6$ | $1.19 \times 10^6$ | |

Contact for 30 minutes.

Time 0 minutes: $<10^2$ on each medium.

Time 30 minutes: as in Table 1.

Platings on MM *leu str* and on MM *pro str* served a control of recombination frequencies of the two types of biparental crosses Hfr × F⁻ occurring in each mixture.

In Table 3, numbers of recombinants from noninterrupted samples are given for the same three types of crosses as in Figure 1. It can be seen that in this case triparental progeny are already present in the mixture at ten minutes. A gradual beginning was always observed both for interrupted and noninterrupted experiments, and also in Hfr × F⁻ crosses, probably due to lack of synchronization. In addition it will be noticed that 683 is somewhat less efficient than 682 in conjugation.

These results may assist our problem as follows: firstly, they rule out definitely the possibility of repeated mating in the formation of triparental progeny. Secondly, they suggest that a simultaneous mating of three cells can occur. The synchrony between the kinetics of M and $C_2$ in Figure 1 seems to support the idea that when the later marker (*leu*⁺) begins to be inoculated into the F⁻, the first one (*pro*⁺) is already present in a fairly high proportion of zygotes. In other words, if injection by the two males could not be simultaneous, we should expect a lag between time of entry of the later marker and time of appearance of triparental progeny. These considerations, together with the early presence of triparental recombinants in noninterrupted samples of Table 3, support a one-stage process.

To test the second possibility, the following experiment has been performed (Figure 2):

The F⁻ was allowed to mate separately with each of the two Hfr's for 30 minutes, after which time conjugation was interrupted, the second Hfr added, and the rate of appearance of triparental progeny observed from these two mixtures, and from a third one, in which the three parents had been mixed together at the same time as in previous experiments.

Results show that in the two mixtures in which previous contact had occurred between the F⁻ and one of the Hfr's, triparental recombinants reach higher values sooner than in the case where mixing had been simultaneous. Values

TABLE 3

*Kinetics of conjugation of a* "ménage à trois"

| Selective medium | Time of contact (minutes) 0 | 10 | 20 | 30 | 40 | 50 | 60 |
|---|---|---|---|---|---|---|---|
| MM *str* (M)* | <20 | $1.3 \times 10^3$ | $2.8 \times 10^3$ | $5.2 \times 10^3$ | $4.3 \times 10^4$ | $4.3 \times 10^4$ | $6.2 \times 10^4$ |
| MM *str leu* ($C_1$)* | <20 | $7.5 \times 10^4$ | $1.1 \times 10^6$ | $2.5 \times 10^6$ | $4.2 \times 10^6$ | $7.0 \times 10^6$ | $7.8 \times 10^6$ |
| MM *str pro* ($C_2$)* | <20 | $4.7 \times 10^4$ | $6.5 \times 10^5$ | $1.2 \times 10^6$ | $1.8 \times 10^6$ | $4.4 \times 10^6$ | $7.0 \times 10^6$ |

* Symbols as in Figure 1.

Mating mixture: F⁻ = 439 : $3.9 \times 10^8$
Hfr *leu* = 682 : $3.4 \times 10^8$
Hfr *pro* = 683 : $4.3 \times 10^8$
} viable cells/ml

Time 0 minutes: interrupted on a tenfold dilution, of which 0.5 ml were plated (this was obviously the same 0 minutes sample of Figure 1).

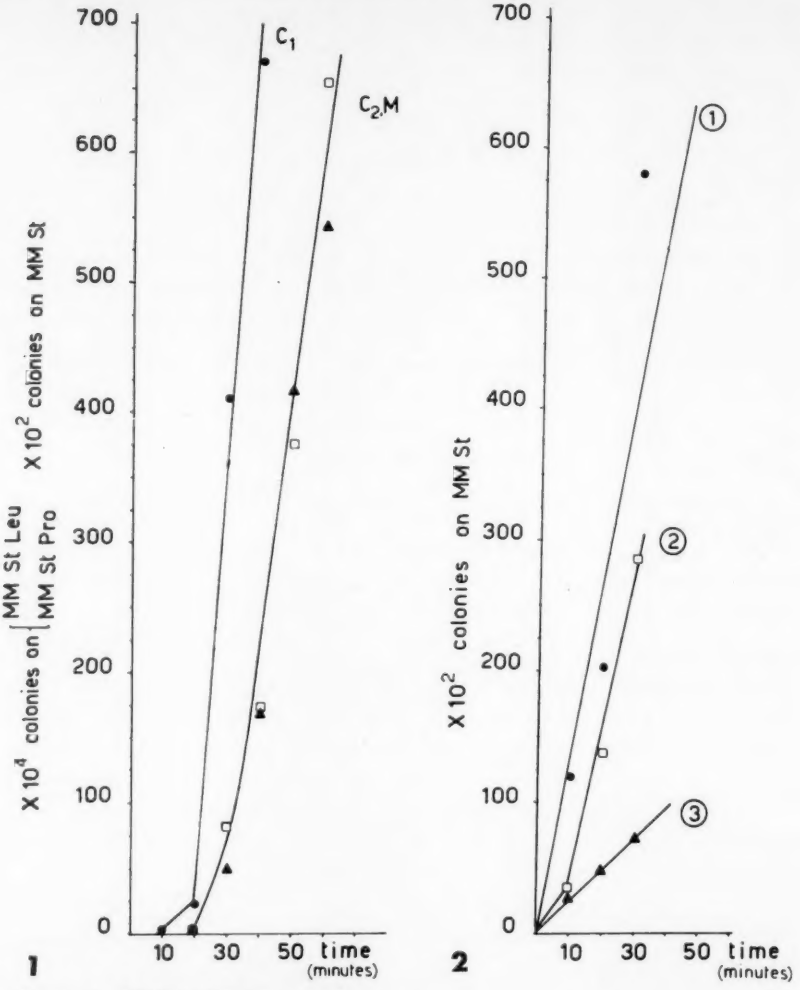



FIGURE 1.—Mating mixture:

$F^-$ = 439 : $3.9 \times 10^8$
$Hfr_1$ *leu* = 682 : $3.4 \times 10^8$ } viable cells/ml.
$Hfr_1$ *pro* = 683 : $4.3 \times 10^8$

Samples interrupted on a tenfold dilution.

● = $C_1$, recombinants between 439 and 682 (platings on MM *str leu*) = entry of *pro*$^+$.

□ = $C_2$, recombinants between 439 and 683 (platings on MM *str leu*) = entry of *leu*$^+$.

▲ = M, triparental recombinants (platings on MM *str*).

A single line has been drawn to connect both □ and ▲ points since their kinetics seem the same, although ▲ frequencies are 1/100 of □ ones.

varied with the Hfr secondarily added, which can be related to the slower rate of conjugation of 683, seen in Table 3. At 60 minutes, approximately the same frequencies are reached in the three mixtures.

In analyzing these data, we can assume that in mixtures 1 and 2 there is already a high number of diploid zygotes at time zero minutes of the experiment. These would be ready for the second "stage" of mating (with the second Hfr), thus increasing, in early times, the rate of formation of triparental zygotes.

From these results we can conclude that both the two mechanisms postulated are probably involved in the formation of triparental progeny.

*Effect of F⁻ in Hfr × Hfr crosses:* We noted from data of Table 1 that the proportion of recombinants originating from Hfr × Hfr matings in a triparental mixture was higher than expected. Usual values for recombination frequency between two Hfr's are about $10^{-3}$ or less (CAVALLI-SFORZA, LEDERBERG and LEDERBERG 1953). We confirmed this for our strains, and simultaneously found that the frequency increased when a female was added to the Hfr × Hfr mixture (Table 4). This happened without the female taking part in recombination but for the few triparentals which were scored by plating on MM *str*. The latter, as we have seen, occur with a frequency a hundred times lower, and are therefore not involved.

Nevertheless, colonies grown on MM were scored again by streaking on streptomycin; and again only one percent of them was found $str^r$.

No "mating-activating substance" could be demonstrated in the cell-free supernatants of the female or of the triparental mixture treated as in crossing experiments. A similar effect (only slightly reduced in size) in increasing recombination frequencies between males was observed adding UV killed females (survival $10^{-7}$) to Hfr × Hfr mixtures (Table 5). It is known that UV killed females cannot participate in a cross. It was thought, therefore, that the F⁻ might act in some way as an "attraction centre". For instance, a more effective pairing of the two Hfr's when a female is present could be accounted for by a difference in the surface properties between the males and the females

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FIGURE 2.—● = Mixture (1): 439+683 were previously incubated together for 30 minutes at 37°C, after which time conjugation was interrupted, and 682 added = time 0 of the experiment. Before adding 682, the interrupted mixture was titrated for recombinants between the preincubated parents (diploid zygotes) on MM *pro str*. These were $0.87 \times 10^6$.

□ = Mixture (2): 439+682 treated as 439+683 in mixture (1). 683 added at time 0 of the experiment. Recombinants between 439 and 682 after preincubation and interruption (time 0 of the experiment): $0.92 \times 10^6$.

▲ = Mixture (3): 439+683+682 mixed contemporaneously at time 0 of the experiment.

At 60 minutes the same level of about $2 \times 10^5$ was reached in the three mixtures.

Concentration of strains in the three mixtures:

$$\left.\begin{array}{l} F^- = 439 : 1.9 \times 10^8 \\ Hfr_1\ leu = 682 : 1.4 \times 10^8 \\ Hfr_1\ pro = 683 : 0.9 \times 10^8 \end{array}\right\} \text{viable cells/ml.}$$

TABLE 4

*Effect of F⁻ on recombination frequencies of Hfr × Hfr crosses*

| Selective medium | Viable cells/ml | (I) 682: 1.5×10⁸ U 187:1.65×10⁸ alone | (I) +439:0.84×10⁸ | (II) 684: 2.1×10⁸ 683:1.25×10⁸ alone | (II) +439:0.84×10⁸ | (III) 682: 1.5×10⁸ 683:1.25×10⁸ alone | (III) +439:0.84×10⁸ | (IV) 684: 2.1×10⁸ Hfr *leu* U 187:1.65×10⁸ Hfr *pro* alone | (IV) +439:0.84×10⁸ F⁻ |
|---|---|---|---|---|---|---|---|---|---|
| MM | | $5.1 \times 10^5$ | $6.8 \times 10^6$ | $2.7 \times 10^5$ | $4.6 \times 10^6$ | $1 \times 10^5$ | $1 \times 10^7$ | $1.35 \times 10^6$ | $4.05 \times 10^6$ |
| MM *str* | | | $3.6 \times 10^4$ | | $3.9 \times 10^4$ | | $1.7 \times 10^5$ | | $1.3 \times 10^4$ |
| MM *str leu* | | | $5.9 \times 10^6$ | | $4.0 \times 10^6$ | | $4.3 \times 10^6$ | | $2.2 \times 10^6$ |
| MM *str pro* | | | $2.9 \times 10^6$ | | $5.2 \times 10^6$ | | $3.7 \times 10^6$ | | $2.0 \times 10^6$ |
| Increase factor due to F⁻ in r.f. of Hfr × Hfr | | | 13 | | 17 | | 100 | | 3 |
| Hfr × Hfr in triplets (*qr*) | | | $2.15 \times 10^{-2}$ | | $1.4 \times 10^{-2}$ | | $3.6 \times 10^{-2}$ | | $1.1 \times 10^{-2}$ |
| Triparentals ($qr^2$) | | | $4.3 \times 10^{-4}$ | | $4.6 \times 10^{-4}$ | | $20 \times 10^{-4}$ | | $1.5 \times 10^{-4}$ |

Contact for 30 minutes. Dilutions without interruption. Platings on MM reveal Hfr×Hfr progeny both in mixtures with and without F⁻ added. Platings on MM *str* from F⁻ containing mixtures give frequencies of triparental matings. Platings on MM *str leu* and MM *str pro* give the two biparental Hfr×F⁻ recombination frequencies. In the two bottom lines relative frequencies are calculated for Hfr×Hfr and triparental progeny in the mixtures with F⁻ added (see discussion).

TABLE 5

*Effect of UV killed F⁻ on recombination frequencies of Hfr × Hfr crosses*

| Hfr's | Alone | + F⁻ (alive) | + F⁻ (UV killed) |
|---|---|---|---|
| 682 × 683 | $4 \times 10^4$ | $1.4 \times 10^6$ | $8.3 \times 10^5$ |
| 682 × U 187 | $8 \times 10^4$ | $3.2 \times 10^6$ | $1.2 \times 10^6$ |

Contact for 30 minutes. Dilutions without interruption. Platings on MM.

(MACCACARO and COMOLLI 1956; ØRSKOV and ØRSKOV 1960; SNEATH and LEDERBERG 1961), both Hfr's being attracted by the F⁻. The correlation which seems to exist between the degree of increase in recombination frequency of Hfr × Hfr in presence of F⁻, and the frequencies of triparental recombinants, respectively, in the four different mixtures of Table 4, might support this idea.

## DISCUSSION

We have seen that the kinetics of markers injection and of conjugation, in a triparental cross, support the hypothesis of a role of simultaneous mating of three cells in the formation of triparental recombinants (one-stage process). On the other hand, the process can also take place in two stages, as supported by the experiment of Figure 2. In this case, mating had been allowed between only one of the males and the female for a time (30 minutes) which usually is sufficient for the majority of pairs to form, and, after that, mating was interrupted. We can assume that at this time most competent F⁻ cells were converted into merozygotes, separated from the fertilizing male. Addition of a second male would result (A) in a decreased outcome of triparental recombinants if the zygotes had become immune to further matings; or (B) in a faster rate of appearance of triparental progeny if zygotes are not immune, for, in this case, they will be ready for the "second stage" of the process. What was actually found is in agreement with alternative (B).

No evidence of interference against triparental matings was found from other sources either. In the absence of any complication, the expected relative frequency of triparental matings could be estimated as the product of the relative frequencies of the two biparental Hfr × F⁻ crosses. Actually it should be a little smaller, since biparental progeny can arise from pairs as well as from triplets (or larger clumps). One could calculate a "coincidence" value, i.e. the ratio of the observed to the expected frequency of triparentals. (The relative frequency of recombinants was calculated as the ratio of the total number of recombinants to the F⁻ cells input.)

If we calculate this "coincidence" from the data of Tables 1–4, we find, for triparental recombinants, an average value of nearly 1. The range of individual "coincidence" values is from 3 to 0.25, and this variation could be justified by the magnitude of the experimental errors involved.

The inference could be made, therefore, that on the average, there is neither

immunity nor other phenomena making triparental matings more or less probable than expected.

Turning to the problem of Hfr × Hfr recombination frequencies, we brought evidence that, in the presence of a female, males can mate between themselves more actively than in a plain Hfr + Hfr mixture. We explained this on the hypothesis that, when a female is present, Hfr × Hfr recombinants arise mostly from triplets (or larger clumps) containing at least one $F^-$ cell, a more effective contact being established between the males by means of attraction exercised by the female. In these conditions, Hfr × Hfr recombination frequencies reach values which are roughly as high as those of Hfr × $F^-$ crosses. Let us verify this on data of Table 4. Having established that, on the average, there is no interference against triparental matings, we may compare relative frequencies of Hfr × Hfr progeny with relative frequencies of triparental ones. Both these types of recombinants arise from triplets or larger clumps. Relative frequencies of Hfr × Hfr progeny (calculated as the ratio: concentration of recombinants on MM/sum of concentrations of parental Hfr's in the mating mixture) and those of triparental progeny (recombinants on MM *str*/parental $F^-$ in the mating mixture, the $F^-$ being in this case the limiting factor) are given in the last two lines of Table 4, respectively. If we call $r$ the probability of recombination *after effective contact has been established* between the cells, and if $r$ is the same for any recombinational event, i.e. Hfr × Hfr or Hfr × $F^-$, the two values just mentioned above should be equal to $qr$ and $qr^2$, respectively, $q$ being a factor accounting for the proportion of clumps in which both Hfr's and one $F^-$ are represented. If $q$ is high, e.g. between 0.5 and 1—and several considerations make it likely—then one would expect that the two frequencies of Hfr × Hfr, and Hfr × Hfr × $F^-$, are approximately given by $r$ and $r^2$. In fact, the latter value (see Table 4) is found to be approximately the square of the former.

The idea is thus confirmed that the low value of recombination frequency usually found in Hfr × Hfr crosses depends, mostly, on a difficulty in the first stage of mating, namely effective contact between cells; and that once contact has been established, recombination efficiency is about the same as in Hfr × $F^-$ crosses.

Another possible explanation is that the $F^-$ cells "devirilize" (SNEATH and LEDERBERG 1961) part of the Hfr cells; devirilized Hfr's might then more easily mate with the residual normal Hfr cells. Experiments are under way which may throw light on this possibility.

## SUMMARY

Occurrence of triparental recombinants between two Hfr and one $F^-$ has been demonstrated in *E. coli* K-12. These are found with a frequency of about $10^{-4}$ in a mating mixture (*ménage à trois*) in which progeny from normal Hfr × $F^-$ crosses appear at about $10^{-2}$. The possibility of triparental recombination is independent of the Hfr type, occurring both with different and with similar Hfr. Formation of triparental zygotes is probably accomplished both by a simul-

taneous mating of three cells and by further matings of biparental zygotes with a second male. The latter mechanism implies a lack of "immunity" of an already fertilized $F^-$. An effect of the female in increasing Hfr × Hfr recombination frequency has been observed and explained on the assumption that females act as attraction centers.

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# GONADAL MOSAICISM AS A FACTOR IN DETERMINING THE RATIO OF VISIBLE TO LETHAL MUTATIONS IN DROSOPHILA[1]

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AS is well known, when sperm cells of Drosophila are treated with X-rays, relatively few of the induced mutations are visibles; the great majority are lethals. In the case of chemical mutagens, the results are somewhat different. According to work reported by the FAHMYS (1956) chemical mutagens induce a relatively high visible rate. The FAHMYS have concluded that chemical mutagens have a specific effect on visible loci. However, CARLSON and OSTER (1961) have suggested that the high visible rate in question might be associated with gonadal mosaicism. The present studies confirm this suggestion.

## METHODS

Mutations, both visible and lethal, were detected by means of MULLER's Maxy technique. In this technique, the females are heterozygous for an X chromosome which contains recessives at 14 visible loci. The males have the normal alleles at these loci. Because of balanced lethals, the only $F_1$ females which survive are those which receive the recessive chromosome of the mother and the treated chromosome of the father. Accordingly, mutations arising in the paternal X at any of the 14 visible loci under study show in the $F_1$ females, either as whole-body mutations or fractionals. In the event that the paternally derived (treated) chromosome of an $F_1$ female contains a lethal, this female produces no sons (as in the *CIB* method), because males with the recessive X are inviable, and those which receive a treated X with a lethal also are.

In scoring for visible mutations at 14 selected loci by the Maxy technique, the personal factor, ordinarily involved in detecting visibles, is avoided. However, since the entire X is scored for lethals (not just 14 loci), the ratio of visibles to lethals is lower than usually reported when the visibles are not restricted to 14 loci.

Four series of experiments were run. In the first, the males received 3000r X-rays. In the remaining three, the males received an intra-abdominal injection of a chemical mutagen (2,5 bis-ethyleneiminohydroquinone, phenylalanine mustard, and dimethyl myleran). In each series, the $F_1$ females were examined for visible mutations (both whole-body and fractional). Those which showed

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mutant traits were further bred to verify them as mutants at the visible loci under study. Samples of $F_1$ females of nonmutant appearance were further bred to test for lethals. The samples were derived from numerous $P_1$ males in each series.

## RESULTS

It was found that the ratio of lethals to visibles was much higher in the X-ray series than in any of the chemical series; namely, 80 lethals:1 visible for X-rays as compared with 20:1 for 2,5 bis-ethyleneiminohydroquinone, 13:1 for phenylalanine mustard and 9:1 for dimethyl myleran (Table 1). However, among the visibles the proportion of fractional to whole-body mutations was much lower in the X-ray series than in any of the chemically treated; namely, seven percent fractionals in the X-ray series, as compared with 30 percent, 52 percent, and 67 percent for 2,5 bis-ethyleneiminohydroquinone, phenylalanine mustard, and dimethyl myleran, respectively (data previously reported, ALTENBURG and BROWNING 1961). Moreover, within the chemical series, there was the same sort of inverse relation as just noted between the proportion of lethals and visibles produced by the agent and the percent of fractionals (of total visibles) it produces—a lethal to visible ratio of 20:1 going with 30 percent fractionals in the case of 2,5 bis-ethyleneiminohydroquinone, 13:1 with 52 percent for phenylalanine mustard and 9:1 with 67 percent for dimethyl myleran.

## DISCUSSION

The above results indicate that the higher the percentage of fractionals produced by the agent employed, the lower the ratio of lethals to visibles recovered after treatment with the agent; or *vice versa*, the higher the percentage of fractionals, the higher the ratio of visibles to lethals. Such a correlation would result from gonadal mosaicism and would be expected on theoretical grounds, as suggested by CARLSON and OSTER (1961). For fractional mutations would cause mosaicism, not only of the body as a whole but also of the gonads. Therefore, if a fractional lethal had been induced in the treated X, the $F_1$ female would often

TABLE 1

*Ratio of recessive lethals to visibles and percent of fractionals (among the visibles) produced by four mutagenic agents*

| Mutagenic agent | No. chromosomes tested for lethals | No. (and percent) lethals | No. chromosomes tested for visibles* | No. (and percent) visibles | Ratio of percent lethals to percent visibles | Percent fractionals (among visibles) |
|---|---|---|---|---|---|---|
| X-rays (3000r) | 7,700 | 472(6.14) | 157,000 | 122(.078) | 80:1 | 7 (9 in 122)† |
| 2,5 bis-ethyleneimino-hydroquinone | 1,492 | 29(1.94) | 31,000 | 30(.097) | 20:1 | 30 (9 in 30) |
| Phenylalanine mustard | 3,316 | 41(1.24) | 24,000 | 23(.096) | 13:1 | 52 (12 in 23) |
| Dimethyl myleran | 1,987 | 40(2.01) | 17,000 | 39(.023) | 9:1 | 67 (26 in 39) |

* The data in this column, the next, and the last column were previously reported (ALTENBURG and BROWNING 1961).
† In parentheses: total number of fractional visibles followed by total number of visibles detected.

have a mosaic ovary; i.e., one which was partly normal and partly lethal for the treated X. Such a female would produce some males with the treated chromosome, and in scoring, the X would be misclassified as a nonlethal (since a treated X is scored as a lethal only in the complete absence of males in the $F_2$, as in the C1B method). By contrast, a visible fractional would not be mistaken for a nonmutant, since it would be phenotypically evident in the $F_1$ female that it was mutant. Moreover, the presence of some normal males in the $F_2$ would not cause the treated X to be mistaken for nonmutant; rather, such normal males would confirm the mutation as a fractional, particularly if mutant males were also present. Fractionals would therefore lower the percent of detected lethals, but not the percent of visibles; or *vice versa*, they would increase the proportion of visibles relative to the lethals. (It is being assumed that there is no difference in the percent of fractionals among the lethals and the visibles induced by a given agent.)

Since chemicals induce a higher percent of fractionals than do X-rays, they would expectedly induce a correspondingly higher percent of gonadal mosaics for lethals. Some earlier work reported by AUERBACH (1946) indicated in fact that chemical agents produce gonadal mosaicism for lethal mutations more often (relative to the total lethals) than do X-rays (the experiments having been specially designed for the detecting of gonadal lethal mosaicism). Accordingly, the lowering of the detected lethal rate due to gonadal mosaicism would be greater for chemical mutagens than for X-rays when the usual methods of detecting lethals are employed. The visible rate, relative to the lethals, would be correspondingly increased, and this is actually seen to be the case (Table 1).

The relative visible rate might be expressed in another way than shown in Table 1. As seen from this table, the detected lethal:visible ratio for X-rays is 80:1, and for the first of the three chemicals, 20:1 (or 80:4). The visible rate relative to 80 lethals is therefore four for this chemical as compared with one in the case of X-rays; for the other two chemicals the visible rates (for 80 lethals) are six and nine (Table 2).

Since doses of different kinds of mutagenic agents are considered equivalent if they induce the same percent of lethals, the relative visible rates induced by equivalent doses of X-rays and the three chemicals under study would be regarded as 1, 4, 6, and 9. These results therefore confirm the FAHMYS in showing that chemicals are more effective than X-rays in the induction of visibles, provided we estimate the lethal rates in the usual way. However, in the case of myleran, for example, about two thirds (67%) of the mutations are fractionals. Accordingly, about ⅔ of the lethals are undetected, leaving only about ⅓ which are detected. The actual lethal rate therefore is about three times as high as the detected, making the actual ratio of lethals to visibles 27:1 instead of 9:1. The visible rate, relative to 80 lethals would then be about three instead of nine. Accordingly, the high visible rate with myleran is due mainly to gonadal mosaicism.

However, after making allowance for differences due to gonadal mosaicism, the chemically induced visible rates (i.e., the "corrected") are still about three

times as high as the X-ray induced (Table 2). Perhaps some of the residual difference is due to "masked" mosaics; that is, to $F_1$ females which, for example, have both eyes mutant and which superficially appear to be whole-body mutations, but which have some normal tissue internally. If not more than a small fraction of eggs with the treated X happened to be normal (the rest mutant) such a masked mosaic would throw only a few normal males in the $F_2$, and these might sometimes be overlooked on routine inspection of the culture vial through the glass, particularly in the case of fractional mutations to the darker eye colors, since the normals would not differ very conspicuously from the mutants. In case the $F_1$ female happened to be a mosaic having a corresponding distribution of cells with a lethal and nonlethal treated X, she would produce some males in the $F_2$, and even though there were only a few such males, they would not be so likely to be overlooked. Accordingly, the treated X would be scored as a nonlethal. Masked mosaics would therefore reduce the percent of detected lethals. They are the result of fractionals, just as are the "patent" (phenotypically evident) mosaics, and if their percent were known, it would have to be added to the percent of patent mosaics in estimating the total fractionals produced by an agent.

Assume now that about 25 percent of the fractionals were undetected because they produced masked mosaics. Then, in the case of myleran, about 89 percent of the induced mutations would be fractionals, leaving only about 11 percent which were whole-body. Since the whole-body mutations would be the only ones to yield detectable lethals, the actual lethal rate would be about nine times as high as the detected, making the lethal to visible ratio about 81:1 instead of 9:1. This ratio (81:1) would be about the same as that for X-rays (80:1). Of course there would also be a corresponding proportion of undetected fractionals in the X-ray series, but if we allowed for them, the total fractionals in the X-ray series would be about nine percent (i.e., $4/3 \times 7\%$) instead of seven percent, and this increase would make no appreciable difference in the detected and corrected visible to lethal rates in the present case.

The above percent of masked mosaics was arbitrarily chosen for purposes of illustration, and perhaps it is too high. Possibly some of the difference in the visible rates is real, rather than apparent, in the comparison of X-rays with chemical mutagens. Large deletions would more often express themselves as

TABLE 2

*Apparent and corrected visible rates induced by four mutagenic agents*

| Mutagenic agent | Ratio of detected lethals:visibles | Apparent visible rate (relative to detected lethal rate)* | Percent whole-body mutations | Corrected visible rate (on basis of percent whole-body) |
|---|---|---|---|---|
| X-rays (3000r) | 80:1 | 1 | 93 | 1 |
| 2,5 bis-ethylene-iminohydroquinone | 20:1 | 4 | 70 | 3 |
| Phenylalanine mustard | 13:1 | 6 | 48 | 3 |
| Dimethyl myleran | 9:1 | 9 | 33 | 3 |

* Rate in this and last column expressed in nearest whole number.

lethals than as visibles, and since X-rays are more effective than chemicals in producing large rearrangements, the lethal rate, as compared with the visible, might be somewhat higher with X-rays than with chemicals. However, if we confine ourselves to point mutations, there is no conclusive evidence that chemical mutagens are specific for visible loci in Drosophila.

The close agreement in the corrected visible rates for the three chemical mutagens, as shown in Table 2, would be a very remarkable coincidence if chemicals were specific for visible loci, since their degree of specificity would expectedly differ, as indeed is suggested by their apparent visible rates (4,6,9). However, if there is no such specificity, then the agreement in the corrected rates within the chemical series would be expected. The higher visible rates for the chemicals as a group as compared with X-rays (3 *vs.* 1) might, as above indicated, be due partly to masked mosaics and partly to a difference in the proportion of large rearrangements induced by X-rays and chemicals.

In any event, it is apparent that the results herein reported necessitate a reappraisal of the claim that chemical mutagens are specific for visible loci in Drosophila.

## SUMMARY

The higher the proportion of fractional mutations relative to whole-body produced by treatment of postmeiotic germ cells of the mature Drosophila male with four mutagenic agents, the higher is the ratio of visible mutations to detected recessive lethal mutations. The relative rates are as follows (where percent of fractionals is followed by the ratio of visibles to lethals in parentheses): 3000r X-rays, seven percent (1:80); 2,5 bis-ethyleneiminohydroquinone, 30 percent (1:20); phenylalanine mustard, 52 percent (1:13); dimethyl myleran, 67 percent (1:9). This correlation might be explained (as suggested by CARLSON and OSTER 1961) on the assumption that gonadal mosaicism would lead to a lowering in the percent of detected lethals, since a gonad which was partly normal (and partly lethal) would appear on further testing to be nonlethal. However, the recovered visible rate would not be similarly depressed by gonadal mosaicism, since visible fractionals would be phenotypically evident. These results necessitate a reappraisal of the claim that chemical mutagens are specific for visible loci in Drosophila.

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# RELEASE OF GENETIC VARIABILITY THROUGH RECOMBINATION. VI. DROSOPHILA WILLISTONI

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THE main purpose of experiments on release of genetic variability through recombination is to estimate the amount of genetic variability freed by such a process.

MATHER (1943) and MATHER and HARRISON (1949) pointed out the non-uniformity of chromosomes producing the same phenotypic effects when the character studied is not a major component of fitness. In a series of publications, DOBZHANSKY and his collaborators (DOBZHANSKY 1946; WALLACE, KING, MADDEN, KAUFMANN and MCGUNNIGLE 1953; SPASSKY, SPASSKY, LEVENE and DOBZHANSKY 1958; SPIESS 1959; DOBZHANSKY, LEVENE, SPASSKY, and SPASSKY 1959), measuring a major component of fitness viability, studied the genetic composition of wild-type chromosomes which, when homozygous, gave similar phenotypes, normal to subnormal viability. It turned out that normal chromosomes (showing normal viability when homozygous) can produce through recombination an amazingly large amount of variability, ranging from 20 percent to 85 percent of the variability existing in a natural population. This result was not completely unexpected: CORDEIRO and DOBZHANSKY (1954) had already given evidence that "normal" chromosomes do not constitute a uniform class. These findings are in accord with the hypothesis that the genetic structure of natural populations is more adequately described by the balance hypothesis, rather than by the classical hypothesis, of populations structures (DOBZHANSKY 1955).

The first three articles of this series (SPASSKY *et al.* 1958; SPEISS 1959; DOBZHANSKY *et al.* 1959) have dealt with *D. pseudoobscura, persimilis and prosaltans*. The present paper is concerned with *D. willistoni*. These four species can be divided into two categories. *D. pseudoobscura* and *willistoni* are common, widespread, ecologically versatile species, respectively of the North American and South American fauna. The genetic load in their natural populations is greater than the one found in *D. persimilis* and *D. prosaltans*, which occur in a less extensive area and seem to be ecologically more specialized and less common, respectively in North and South America (DOBZHANSKY and SPASSKY 1953, 1954; PAVAN *et al.* 1951; KRIMBAS 1959). It has already been hypothesized that the two common species have a population structure which is more in accordance with the balance hypothesis than the two less common species. It has also been

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shown that *D. pseudoobscura* releases through recombination a greater amount of genetic variability than *D. persimilis*. Does the same relation exist between the two South American species?

## MATERIALS AND TECHNIQUES

In the three previous papers, samples from two different populations were examined in intra- and interpopulation combinations. In the present paper only one population is examined. This population was collected at Angra dos Reis, Brazil, by PROFESSOR C. PAVAN and his collaborators. Second chromosomes were analysed for viability effects when homozygous (KRIMBAS 1959). Ten chromosomes showing a normal or mildly subvital viability were retained to be used in the present experiment. The experimental technique was as follows. Males, or sons of the females collected in the wild, were crossed to females homozygous for the two second chromosome recessive mutants abbreviated bristles and brown eyes (*abb bw*). A single male from each progeny was crossed to females in which one second chromosome carried *abb bw* and the two dominant mutants Star (*S*) and Hook (*Hk*) and an inversion suppressing crossing over. *S* is lethal in homozygotes. In the next generation males and females *S Hk* non-*abb–bw* were crossed. The percentage of wild type flies in the progeny is a measure of the viability of the chromosomes in homozygous state. Ten such chromosomes showing normal viability were selected. They have been kept either in homozygous condition, or balanced against *S Hk abb bw*. Before the beginning of the present experiment they were reisolated to insure that no lethal mutants had arisen. Following this reisolation, males *S Hk* non-*abb–bw* from one wild type chromosome line were crossed to females *S Hk* non-*abb–bw* from another wild type chromosome line. The 45 possible crosses were made. One or several wild type females from every set of progeny were crossed to *abb bw* males, and ten males coming from each cross were selected. Every such male had one second chromosome bearing *abb bw* and another produced by recombination of parts of two normal wild chromosomes. Each male was crossed to *S Hk abb bw*/recessive lethal females, and *S Hk* non-*abb–bw* males and females from their progeny were inbred. One hundred flies were counted in the progeny of each cross, in order to estimate the viability of each recombinant chromosome in homozygotes. Four hundred and fifty such chromosomes were analysed. At the same time six replicate cultures of the ten original chromosomes were tested: *S Hk* non-*abb–bw* males and females heterozygotes for one and the same wild type chromosome were crossed and 100 flies from each replica culture were counted. Sixty such counts were made. All these previous counts constitute the primary data. All cultures were raised in SPASSKY's medium and at 25°C. The statistical analysis of the data is described in LEVENE (1959) and presented in a similar way as in the three previous papers.

## RESULTS

The ten original chromosomes differed in two inversions (E and F) in their left arms (DOBZHANSKY 1950; DA CUNHA, BURLA and DOBZHANSKY 1950). The

length of inversion E is 15.5 percent of the whole chromosome and the length of F is about 6.0 percent. It is possible, however, that in heterozygotes for both inversions crossing over is mostly suppressed also in the middle region, thus involving in total about one quarter of the length of the chromosome. Table 1 gives their structural constitution and indicates the fertility of males and females homozygous for these chromosomes. With *D. willistoni* being an extremely polymorphic species, as far as chromosome inversions are concerned, it would be difficult to obtain ten structurally identical second chromosomes normally viable in homozygous state. In this respect our experiment is different from the three previous ones. The amount, then, of variability released depends on the structural similarity of the material. What is measured here is the variability released when two randomly taken normal wild type chromosomes recombine, even though they may not be structurally similar.

The original chromosomes were tested three times: once when first isolated from the wild population, another time when reisolated before the experiment, and a third time at the end of the experiment. The figures reported in Table 2 are of this last test. It turned out (Table 3) that between the second and third tests the amount of genetic variability increased considerably. This was due to a decrease of viability of two of the ten chromosomes, namely 100 and 207, which entered entirely the subvital class (Table 3). It seems that genetic alterations took place between the beginning and the end of the experiment. This is why the analysis of our data is done twice. The first analysis includes all the combinations of the ten original chromosomes; the second, the combinations of eight of these (chromosomes 100 and 207 being excluded). In the second case only 280 recombinants are analyzed statistically.

The experimental data are summarized in Table 2. The strain numbers are shown at the top and the left margins of the table. Numbers above and to the right of the diagonal are the mean numbers of wild type flies (non-*S–Hk*) in the test cultures of the ten recombinant chromosomes of every cross. Numbers at the left and below the diagonal give the variances of the viabilities (numbers of wild type flies) in the ten cultures of recombination products of a given pair

TABLE 1

*Gene arrangements in the original chromosomes and fertility of individuals homozygotes for them*

| Chromosome | Structural type | Female | Male |
|---|---|---|---|
| 456 | $E_1$ $F_1$ | Fertile | Fertile |
| 206 | $E_1$ $F_2$ | Sterile | Fertile |
| 205 | $E_1$ $F_1$ | Fertile | Fertile |
| 503 | $E_1$ $F_1$ | Fertile | Sterile |
| 208 | $E_1$ $F_1$ | Fertile | Fertile |
| 100 | $E_2$ $F_2$ | Sterile | Fertile |
| 207 | $E_2$ $F_1$ | Fertile | Sterile |
| 209 | $E_2$ $F_2$ | Fertile | Fertile |
| 204 | $E_1$ $F_1$ | Fertile | Sterile |
| 512 | $E_2$ $F_2$ | Fertile | Fertile |

of original chromosomes. The squares along the diagonal show the mean viabilities above and the variances of the homozygotes for the ten original chromosomes below. These variances and viabilities refer to the third test of the original chromosomes at the end of the experiment. Their variances are residual variances obtained by subtracting the binomial sampling error from a crude variance. This is why some of these variances are negative. This means that the variance estimated is not statistically different from zero. On the contrary the variances of viabilities of each one of the 45 combinations indicated at the left and below the diagonal are crude variances. The reason is that the environmental and binomial

TABLE 2

*Viability (above and to the right of the diagonal line) and variance (below and to the left of the diagonal) of the recombination products of the ten original chromosomes (456, 100, . . . , 206)*

| | 456 | 100 | 205 | 503 | 208 | 207 | 209 | 204 | 512 | 206 | $\bar{M}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 456 | 39.3<br>+5.2 | 31.2 | 43.2 | 33.4 | 41.6 | 39.3 | 33.9 | 42.6 | 41.7 | 37.5 | 38.3<br>39.1 |
| 100 | 175.3 | 19.8<br>+34.7 | 23.0 | 27.9 | 35.9 | 15.2 | 19.4 | 28.5 | 21.4 | 28.7 | 25.7 |
| 205 | 30.0 | 171.1 | 32.3<br>+16.8 | 36.3 | 23.6 | 34.8 | 35.2 | 31.0 | 35.7 | 39.0 | 33.5<br>34.9 |
| 503 | 61.4 | 182.3 | 40.2 | 34.7<br>−6.0 | 38.4 | 36.1 | 38.8 | 39.4 | 42.0 | 36.9 | 36.6<br>37.9 |
| 208 | 102.7 | 134.5 | 296.5 | 50.7 | 42.7<br>−14.2 | 27.1 | 36.2 | 35.6 | 37.6 | 31.2 | 34.1<br>34.9 |
| 207 | 16.9 | 95.1 | 63.7 | 23.2 | 154.3 | 25.2<br>+12.9 | 26.8 | 33.3 | 28.2 | 31.0 | 30.2 |
| 209 | 141.9 | 93.8 | 56.4 | 50.6 | 57.3 | 278.4 | 39.8<br>−1.9 | 31.4 | 35.7 | 36.8 | 22.7<br>35.4 |
| 204 | 36.3 | 60.3 | 318.9 | 38.3 | 67.6 | 88.2 | 161.2 | 40.2<br>−2.7 | 41.5 | 41.7 | 36.1<br>37.6 |
| 512 | 117.8 | 222.5 | 260.4 | 51.6 | 61.4 | 226.0 | 134.7 | 37.4 | 38.8<br>−10.4 | 33.7 | 35.3<br>38.3 |
| 206 | 23.6 | 262.5 | 38.2 | 86.8 | 312.2 | 378.4 | 191.1 | 30.9 | 77.1 | 39.3<br>−12.8 | 35.2<br>36.7 |
| $\bar{U}$ | 78.4<br>73.4 | 155.3 | 141.7<br>148.7 | 65.0<br>54.2 | 137.5<br>135.5 | 147.1 | 129.5<br>113.0 | 93.2<br>98.6 | 132.1<br>105.8 | 155.6<br>108.6 | |

Strain numbers shown at top and left margins. For further explanations see text.

components of the total variance are different when the analysis is done for all chromosomes and when it is done for combinations of only eight original chromosomes. The squares on the last horizontal line and on the right vertical line indicate the means of the variances and of the viabilities of the recombinational products for each chromosome. Where two numbers are shown, the upper figure shows the mean when the analysis includes all the original chromosomes, and the lower indicates the mean value computed for the products of recombination of only eight original chromosomes (excluding chromosomes 100 and 207).

The first observation is that the means of the viabilities of the recombinant chromosomes are generally lower than those of the original ones. This is true in six out of ten cases (when all chromosomes are analysed) and in five out of eight (when only eight original chromosomes are used). Furthermore, the mean value of all the recombinants is $33.76 \pm 0.22$, the mean value of the originals being $34.67 \pm 0.61$ when all chromosomes are used, and $36.84 \pm 0.29$ for recombinants and $38.40 \pm 0.70$ for the originals when only eight original chromosomes are used. These observations are similar to those in the three previous experiments, although the phenomenon of decrease of the mean viability of the recombinants does not seem to be so important here. We should not forget that original chromosomes are a selected sample from a natural population and have a higher viability than the mean viability in the population. Their recombinants tend to restore the variability existing in nature. This implies statistically a decrease of the mean of their viabilities.

The variance produced by differences of viabilities between the six replicate cultures of each original chromosome is due to two causes: environmental differences between replicate cultures and sampling errors. The binomial sampling component is easily estimated. By subtracting the binomial sampling component from the crude variance (total residual variance) of the original chromosomes, we can estimate the variance due to environmental differences. We assume that the same amount of environmental variance is included within the crude variance between recombinants in each cell. We can also estimate, in the case of the recombinants, the binomial sampling component of variance. By subtracting the environmental and sampling components from the crude variance of the recombinants, we obtain the within-each-cross-produced variance. This variance is (especially when the two original chromosomes involved have the same viabilities) the one we are primarily interested in. It is the genetic variance produced by recombination of similar chromosomes. It is equal to 99 when all original chromosomes are considered and to 81 where only eight original chromosomes are used. If we exclude from our calculations chromosomes 100 and 207, which changed in viability during the experiment, we can consider that the within-crosses-estimated variance represents only the genetic variance released by recombination between normal chromosomes. This value is large, much larger, than the environmental variance existing between replicate cultures of the same original chromosome.

The data of the intrapopulational crosses are used for analysis of the variance of the means which is reported in Tables 3 and 4. This analysis is similar to a

diallel analysis of variance. Sums of the squares and mean squares were calculated and used for the F tests which are indicated in Table 4. From the mean squares the components of variance were estimated, which are summarized in Table 3 (LEVENE 1959; GRIFFING 1956; SPASSKY *et al.* 1958). The total variability of the viability is divided in components listed in their hierarchical order: additive genetic variability, nonadditive genetic variability, genetic variability between different crosses which is the sum between the two previous components, genetic variability within the crosses (that due to recombination), total genetic

TABLE 3

*Components of variance of means and of variances*

| Description | Additive between crosses | Nonadditive between crosses | Total between crosses | Within crosses | Total genetic | "Real" residual | Binomial sampling | Total residual |
|---|---|---|---|---|---|---|---|---|
| Means | | | | | | | | |
| Intra for all | 14.5 | 8.0 | 22.5 | 99 | 121.5 | 2.2 | 23 | 25 |
| Intra except 100 + 207 | 0.46 | 7.8 | 8.3 | 81 | 89 | 0 | 24 | 24 |
| Original: all: when first isolated | 2.6 | .. | 2.6 | .. | 2.6 | .. | .. | 13 |
| Original: all: when reisolated | 5.0 | .. | 5.0 | .. | 5.0 | .. | .. | 25 |
| Original: all: end experiment | 51 | .. | 51 | .. | 51 | 2.2 | 22 | 24 |
| Original: except 100 + 207: when reisolated | 0 | .. | 0 | .. | 0 | .. | .. | 25 |
| Original: except 100 + 207: end experiment | 7.5 | .. | 7.5 | .. | 7.5 | 0 | 20 | 20 |
| Variances | | | | | | | | |
| Intra for all | 2.6 | 53 | 56 | 33 | 89 | .. | .. | 1.2 |
| Intra except 100 + 207 | 0 | 60 | 60 | 24 | 84 | .. | .. | 0.92 |
| Original | 0 | .. | 0 | .. | 0 | .. | .. | 2.4 |

TABLE 4

*Analyses of variance*

| Description | d.f. | On means F | On means P, percent | On variances F | On variances P, percent |
|---|---|---|---|---|---|
| Recombinants for all original chromosomes | | | | | |
| Main effect | 9, 35 | 6.74 | <.05 | 1.45 | 25–10 |
| Interaction | 35, 405 | 1.58 | 5–1 | 2.57 | <.05 |
| Int. of int. | 9, 35 | 0.65 | 75–50 | 1.79 | 25–10 |
| Within | 405, 50 | 5.04 | <.05 | 26.2 | ≪.05 |
| Recombinants for all except 100 + 207 | | | | | |
| Main effect | 7, 20 | 1.20 | 99.5–99.9 | 0.84 | 99.5–99.9 |
| Interaction | 20, 252 | 1.75 | 5–1 | 3.46 | <.05 |
| Int. of int. | 7, 20 | 0.56 | 99.9–99.95 | 5.50 | 0.1–0.5 |
| Within | 252, 72 | 5.16 | <.05 | 25.6 | ≪.05 |

variability which is the sum of the two previous components, residual variability divided in real residual (environmental) and in binomial sampling variance.

In order to make the analysis of variance for the means, it was assumed that the true variance within crosses was constant, showing different values only owing to sampling errors. This is not necessarily true. In order to estimate the variability of the variance, an analysis of the variance of variances is made. Every variance is taken as a single number. The additive component of this variability can be estimated as in the analysis of the means. In order to estimate the nonadditive component, we subtract from the mean square for interaction a theoretically calculated sampling variance within the crosses (which is given in LEVENE 1959). It should also be noted that the F value for the main effect was obtained by a nonparametric analysis of variance in order to improve the sensitivity of the estimation. This test is based on ranks which are transformed, in order to show a greater sensitivity, into equivalent normal deviates, as explained in LEVENE 1959.

Two significant figures are generally given in Table 3 for every component of variance. It is clear from Table 4, in the analysis of variance of the means, that the main effect which is statistically significant, when we analyse the recombinants for all original chromosomes, is only due to the influence of chromosomes 100 and 207. When these two chromosomes are excluded, the main effect disappears. Also the correlation between the means of the recombinants and the original chromosomes (when only eight are used) is very small and statistically not different from zero ($\pm 0.02$). On the contrary, the interaction is significant at the five percent level in both cases. This nonadditive component indicates here the epistatic interaction only, the chromosomes being tested in homozygous state. The additive component is also not significant in the analysis of the variance of variances, while the nonadditive component is highly significant in both cases. The within-crosses component seems to be half as large as the total between crosses. The test of interaction for interactions was performed in order to investigate if the nonadditive component of variance was greater when certain chromosomes were involved as one of the partners for the production of recombinants. It is significant only in the analysis of variance of variances where only eight original chromosomes are involved. The mean square interaction for different chromosomes varies, and this variation is greater than the one expected by chance, so that some of the chromosomes are giving less predictable results than others. The analysis of our results, especially when the two chromosomes 100 and 207 are excluded, thus gives the same picture as the one presented in the three previous publications.

As in the previous papers, we observed some chromosomes which, although they were recombinational products of two normal or sometime subvital chromosomes, were lethal when homozygous. Such chromosomes have been first described by DOBZHANSKY (1946), and later on found repeatedly by him and his co-workers. They were called synthetic lethals on the assumption that their lethality was not due to point mutation but to epistatic interactions between blocks of genes within the chromosomes. HILDRETH and GANTNER, trying to

verify these findings, failed to obtain synthetic lethals. This can partly be explained (for a critical review, KRIMBAS 1960). Recently DOBZHANSKY and SPASSKY (1960), by desynthesis of synthetic lethals, proved their existence in *D. pseudoobscura*. In our material, 21 out of 450 chromosomes were lethals when homozygous, that is to say 4.7% ± 1.0%. Ten out of these 21 were produced in crosses with the participation of the chromosomes 100 or 207. Out of 280 recombinant chromosomes produced by the remaining eight original chromosomes, which did not deteriorate during the experiment, 11 were lethals when homozygous, that is to say 3.9% ± 1.0%. These two percentages are not statistically different. (The catalogue of the obtained lethals is given in Table 5.)

The lethality of these chromosomes could be produced either by mutation or by gene interaction. In our experiment it is difficult to decide rigorously between the two alternatives, although synthetic lethality is the most probable explanation. The tendency of such lethals to occur in certain crosses should be emphasized. Thirty-one percent of the 45 crosses gave at least one synthetic lethal (and 25 percent of the 28 crosses if we exclude chromosomes 100 and 207). It should be noted that the percentage of the synthetic lethals in *D. willistoni* is smaller than might have been expected. *D. pseudoobscura* produced 4.9 percent synthetic lethals and 20 percent of its crosses produced at least one lethal. These figures are respectively 2.3 percent and 8.4 percent in *D. persimilis* and 5.7 percent and 18 percent in *D. prosaltans*.

Some of the recombination products of chromosomes 100 and 206, when homozygous, gave sterile males, although both of these original chromosomes reduced the fertility of the females only. Also some of the recombination products of chromosomes 100 and 209, when homozygous, gave fertile females but sterile males. These results are reported in detail in KRIMBAS (1960). It is rather difficult, although not impossible, to attribute this male sterility to point mutation. Synthetic sterility, a phenomenon analogous to synthetic lethality, should be recognized.

Recombination products of chromosomes 100 and 209, as well as of chromosomes 207 and 100, which showed a relatively poor viability in homozygotes, showed also an abnormal sex ratio, producing more male than females (Table 6). Some of the females produced had abnormal wings and died very young. The

TABLE 5

*Numbers of synthetic lethal chromosomes obtained in different crosses*

| Cross | Number of lethals | Cross | Number of lethals |
|---|---|---|---|
| 100 × 206 | 1 | 204 × 205 | 2 |
| 100 × 209 | 1 | 206 × 209 | 1 |
| 100 × 503 | 1 | 512 × 205 | 1 |
| 100 × 512 | 2 | 512 × 206 | 1 |
| 207 × 209 | 2 | 208 × 205 | 3 |
| 207 × 512 | 1 | 208 × 206 | 2 |
| 207 × 206 | 2 | 204 × 209 | 1 |

original chromosomes did not show such effects. Genes located on the second chromosome of *D. melanogaster* have been described, which in homozygotes produce similar effects (REDFIELD 1926; BELL 1954).

## DISCUSSION

Table 7 compares the results in *D. pseudoobscura, persimilis* and *prosaltans*, as well as those in *D. willistoni*. It should be pointed out that *D. pseudoobscura* is fully comparable only with *D. persimilis:* the chromosomes studies in these two species are homologous. The same is true of *D. willistoni* and *D. prosaltans*. The most meaningful comparison is therefore the percentage of the variability

TABLE 6

*Abnormal sex ratios in homozygotes for some recombination products*

| | Number of flies | | | |
|---|---|---|---|---|
| | Wild type | | *S Hk* non-*abb–bw* | |
| Chromosome | Male | Female | Male | Female |
| 100 × 209 V | 124 | 39 | 528 | 507 |
| 100 × 209 XVIII | 144 | 52 | 796 | 857 |
| 207 × 100 VI | 20 | 2 | 235 | 237 |

TABLE 7

*Variances of homozygotes for chromosomes of the four species. "Natural" refers to samples of wild chromosomes, and "recombination" to recombination products of pairs of quasi-normal chromosomes obtained from the wild*

| Species and chromosomes | All chromosomes | Quasi-normals | Recombination Natural variance (percent) All chromosomes | Quasi-normals |
|---|---|---|---|---|
| *D.pseudoobscura* | | | | |
| Natural | 140 | 65 | | |
| | | | 43 | 74 |
| Recombination | 60 | 48 | | |
| *D.persimilis* | | | | |
| Natural | 110 | 60 | | |
| | | | 24 | 27 |
| Recombination | 26 | 16 | | |
| *D.willistoni* | | | | |
| Natural | 290 | 20 | | |
| | | | 34.5 | "100" |
| Recombination (all) | 100 | 33 | | |
| | | | 28 | "100" |
| Recombination (except 100+ 207) | 80 | 28 | | |
| *D.prosaltans* | | | | |
| Natural | 200 | 85 | | |
| | | | 25 | 28 |
| Recombination | 50 | 24 | | |

existing in a natural population which is released by recombination between normal chromosomes. *D. willistoni* when all original chromosomes are used releases 34.5 percent of the total variability existing in a natural population and 28 percent if only eight original chromosomes are used. On the other hand, when we deal only with the variability of quasi-normal chromosomes, the variability released by recombination exceeds the one found in nature, but this could be due to the fact that both estimates of variance (natural and recombinational) involve considerable errors. This is why it has been indicated as "100 percent". It seems that *D. willistoni* releases more variability than *D. prosaltans*, exactly as expected according to the balance hypothesis of population structure. In general, these experiments give strong support to this hypothesis.

The only point which remained unexplained, at least in the three previous publications using *D. pseudoobscura*, *persimilis* and *prosaltans*, and which apparently contradicted the balanced hypothesis is the following. Chromosomes coming from the same population should be more similar, as far as their gene contents are concerned, than chromosomes derived from different populations. Different populations are expected to have different gene pools, and generally to contain different alleles of many loci. On this assumption we should expect more variability to be released from interpopulation recombinations than from intrapopulation ones. This has not been observed, however, in *D. pseudoobscura*, *persimilis* or *prosaltans*. L. VAN VALEN (personal communication) suggests the following tentative explantation of these observations: "It is plausible to expect an appreciable amount of selection in nature for irregular interlocus nonadditivity, since this would be an additional means, roughly analogous to balanced polymorphism, of concealing genetic variability from the onslaughts of selection. The recombination experiments in fact suggest that this occurs. This selection will of course be mainly intrademe, as distant populations are known to evolve more or less independently on the level of genotypic integration. Therefore it is to be expected that less additive genetic variance will be shown by intrademe crosses than by interdeme ones, if the demes are distant. Therefore a greater variance is to be expected from intrademe recombination than would result from an additive model. This could easily be enough to counterbalance the difference between the two demes since the selection for nonadditivity would operate in each independently and presumably not identically".

Our experiment has produced some evidence of mutation in the generation of a genetic variability during the experiment. We were obliged, in order to avoid including this variance generated by mutation, to exclude two original chromosomes, which apparently deteriorated, and to analyse the data a second time. It is unknown how much variability can be produced in a quantitative character by mutation, especially when this character is a major component of fitness. The only estimates available deal with such characters as abdominal bristle numbers (DURRANT and MATHER 1954; CLAYTON and ROBERTSON 1955; PAXMAN 1957; BUZZATI-TRAVERSO and SCOSSIROLI 1959; SCOSSIROLI and SCOSSIROLI 1959). It would be extremely useful to obtain such estimates and to deal not only with lethal mutations, but with the entire array of variability. Experiments are being

planned to estimate by the accumulation technique the amount of variability produced in different chromosomes by polygenic mutation in a character such as viability.

It may finally be pointed out that the balanced theory of population structure has not only been able to give a logical account of already known phenomena but has proved very fertile in stimulating experimental work on totally, until then, unexplored fields.

## SUMMARY

The amount of genetic variability released by recombination of "wild-type" chromosomes has been estimated in *Drosophila willistoni*. This estimate is compared to analogous ones in *D. pseudoobscura*, *persimilis* and *prosaltans*. It looks as if common and widespread species of Drosophila (which carry generally heavier genetic loads) release more genetic variability than less common and ecologically more specialized ones. This observation is in agreement with an earlier formulated hypothesis that the population structure of common species is better described by the balanced theory than by classical theory of population structure. Data concerning synthetic lethality, sterility, and polygenic mutation are reported.

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# TWO TYPES OF DOSE-RATE DEPENDENCE OF RADIATION-INDUCED MUTATION RATES IN SPERMATOGONIA AND OÖGONIA OF THE SILKWORM[1]

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ACCORDING to the classical theory of radiation genetics, it seemed to be well established that induced mutation rates do not depend upon radiation intensity. However, in 1958 RUSSELL, RUSSELL and KELLY published their data on mouse spermatogonia showing that chronic $\gamma$-irradiation is mutagenically less effective than acute X-ray irradiation. Soon after this a similar type of dose-rate dependence of radiation-induced mutation rate was confirmed also for the oöcytes of young adult mice (RUSSELL, RUSSELL and CUPP 1959). Since then it has been of great concern whether the same principle holds true for organisms other than the mouse. Evidence consistent with the results obtained on mice has been found for Drosophila oögonia by OSTER, ZIMMERING and MULLER (1959), while the reaction of the spermatogonia of the same insect was less clear.

We have been carrying out, since 1959, a similar study with the silkworm, whose germ cells develop approximately synchronously. The use of this material made it possible to test definite stages of germ cell development for dose-rate dependence of mutation rate. Unexpectedly to us, the results of the first experiment did not agree with the above findings of RUSSELL *et al.;* i.e., mutagenic effectiveness was lower at acute irradiation than at chronic irradiation not only in spermatogonia but also in oögonia. After additional experiments, we found that the mutagenic effectiveness of acute irradiation varies with the stage of the irradiated germ cells, and that in the silkworm a phenomenon in agreement with RUSSELL's finding on the lower effectiveness of chronic irradiation relative to acute did occur at the stage of primordial germ cells. Furthermore, the findings of our first experiment turned out not to be contradictory to those of RUSSELL, for, although we did not know this at the time, RUSSELL, RUSSELL and KELLY (1959) had also reported a second, inverse, type of dose-rate effect in experiments in which a single high acute dose of radiation gave a lower mutation rate than when the same dose was fractionated.

## MATERIALS AND METHODS

For the estimation of mutation rates the specific locus method was used, with the egg color mutants *pe* (pink egg, V–0.0) and *re* (red egg, V–31.7) as markers.

[1] Contribution No. 377 from the National Institute of Genetics, Japan.

Both sexes of a wild-type strain, C108, were exposed to X-rays or $Cs^{137}$ γ-rays of high dose-rate or to $Co^{60}$ γ-rays of low dose rate, at a definite stage or during a definite period in early larval stages as shown in Figure 1. As illustrated in the same figure, throughout the period of the experiment the germ cells are mostly at the stage of spermatogonia, although they continue to develop as the larvae go on growing. Total doses given were 950r in Expt. 594 and 1000r in Expt. 601, 602 and 603. The dose-rate ratios between acute and chronic irradiation for the four sets of experiments were ca. 7600:1, 2950:1, 2500:1 and 2500:1, respectively. Radiation dosage was measured by Victoreen condenser chambers and was checked by small silver phosphate glass rods placed together with the silkworms. Over-all precision of the reproducibility of the exposure doses given to the silkworm was about ± 5% for $Co^{60}$ γ-rays and X-rays and about ± 2% for $Cs^{137}$ γ-rays. Irradiated individuals of the wild-type strain were mated to partners of the marker strain, homozygous for *pe* and *re*. The examination of mutants was carried out 30–40 days after egg laying with the exception of Expt. 602 in which counting was performed 20 days after egg laying. The number of detected mutants was recorded separately for each batch of eggs from an individual mating; a batch contained ca. 500 eggs when the mother was wild type and ca. 320 when she was a double recessive. Data from separate batches were pooled in order to calculate the over-all mutation frequencies. Mutants that appeared in clusters were included individually in the total, since a theoretical consideration leads us to the view that each mutant of a large cluster should be counted in the same way as a single mutant in a batch (KONDO 1961).

For each observed mutation frequency, the 95 percent confidence intervals were calculated according to the statistical tables of FISHER and YATES (1953).

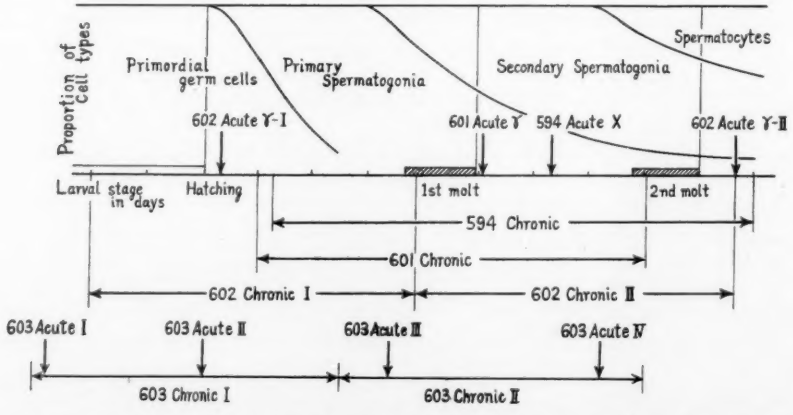


FIGURE 1.—Schematic illustration of irradiation time in each group in relation to the developmental stages of the silkworm larvae and the proportion of different types of germ cells in the testis.

## RESULTS

*The first experiment (Expt. 594):* In the first experiment acute X-ray irradiation was administered in one exposure of 1.48 minutes on the second day of the second instar, while in the chronically irradiated group exposure was given as γ-rays for 189.5 hours during the period from the second day of the first instar to the second day of the third instar, with short intermissions for feeding. The results are shown in Table 1.

Dose-rate dependence of induced mutation rate is striking. However, the results were in complete contrast to those obtained for the mouse (RUSSELL, RUSSELL and KELLY 1958) and for Drosophila (OSTER, ZIMMERING and MULLER 1959). Both for spermatogonia and oögonia the induced mutation rates were clearly lower after acute than after chronic irradiation.

*The second experiment (Expt. 601):* The second experiment was planned to confirm the results of Expt. 594. Since cytological observation had shown that the differentiation of spermatocytes takes place in the testes of the third instar larvae, the chronic exposure was so adjusted that it was terminated before the second molt (Figure 1). $Cs^{137}$ γ-rays were used for acute irradiation in this experiment instead of X-rays as in Expt. 594. The results are shown in Table 2.

The mutation rates in the acutely irradiated group were approximately the same as in Expt. 594 in spite of the change in radiation quality (Expt. 594: X-rays, 634r/min; Expt. 601: $Cs^{137}$ γ-rays, 320r/min). In the chronic group, however, the mutation rates decreased to an appreciable extent, except for *pe* in irradiated males, even though the radiation dose rate was kept nearly the same (Expt. 594: 5.01r/hr; Expt. 601: 6.49r/hr). As a result, the differences in mutation rates between the two exposure groups, acute and chronic, became smaller than in Expt. 594, although the same trend was observed. The shift of the

TABLE 1

*Results of Expt. 594*

| | Total no. observed | Mut. detd. | *pe* Freq. | Induced rate/r | Mut. detd. | *re* Freq. | Induced rate/r | Confidence interval for freq. *pe* | Confidence interval for freq. *re* |
|---|---|---|---|---|---|---|---|---|---|
| | | | $\times 10^{-5}$ | $\times 10^{-7}$ | | $\times 10^{-5}$ | $\times 10^{-7}$ | | |
| a. Irradiation of spermatogonia *pe re* ♀ × ++ ♂ | | | | | | | | | |
| Acute-X | 137,045 | 82 | 59.8 | 4.86 | 22 | 16.0 | 1.57 | 47.6– 74.3 | 10.0– 24.3 |
| Chronic-γ | 105,072 | 86 | 81.8 | 7.18 | 56 | 53.3 | 5.49 | 65.5–100.9 | 40.3– 69.2 |
| Control | 184,111 | 25 | 13.6 | ... | 2 | 1.1 | .. | 8.2– 20.0 | 0.1– 3.9 |
| b. Irradiation of oögonia ++ ♀ × *pe re* ♂ | | | | | | | | | |
| Acute-X | 124,841 | 18 | 14.4 | 0.97 | 11 | 8.8 | 0.56 | 8.6– 22.8 | 5.5– 17.8 |
| Chronic-γ | 80,457 | 48 | 59.7 | 5.74 | 17 | 21.1 | 1.85 | 44.0– 79.1 | 12.3– 33.8 |
| Control | 172,719 | 9 | 5.2 | ... | 6 | 3.5 | ... | 2.4– 9.9 | 1.3– 7.6 |

TABLE 2

*Results of Expt. 601*

| | Total no. observed | Mut. detd. | *pe* Freq. | Induced rate/r | Mut. detd. | *re* Freq. | Induced rate/r | Confidence interval for freq. *pe* | *re* |
|---|---|---|---|---|---|---|---|---|---|
| a. Irradiation of spermatogonia *pe re* ♀ × ♂ + + | | | | | | | | | |
| | | | $\times 10^{-5}$ | $\times 10^{-7}$ | | $\times 10^{-5}$ | $\times 10^{-7}$ | | |
| Acute-γ | 177,754 | 96 | 54.0 | 3.30 | 34 | 19.1 | 1.85 | 43.7– 66.0 | 13.2– 26.7 |
| Chronic-γ | 184,398 | 174 | 94.4 | 7.34 | 46 | 24.9 | 2.43 | 80.9–109.5 | 18.3– 33.3 |
| Control | 166,303 | 35 | 21.0 | . . | 1 | 0.6 | . . . | 14.7– 29.3 | 0.0– 3.3 |
| b. Irradiation of oögonia + + ♀ × *pe re* ♂ | | | | | | | | | |
| Acute-γ | 294,960 | 71 | 24.1 | 1.83 | 39 | 13.2 | 0.89 | 18.8– 30.4 | 9.4– 18.1 |
| Chronic-γ | 299,713 | 105 | 35.0 | 2.92 | 50 | 16.7 | 1.24 | 28.7– 42.4 | 12.4– 22.0 |
| Control | 328,059 | 19 | 5.8 | . . . | 14 | 4.3 | . . . | 3.5– 9.1 | 2.3– 7.1 |

exposure period to the earlier stages seems to have been the cause of the decrease of the difference in mutation rates.

As to the cause of the lower mutagenic effectiveness of acute irradiation in the above two experiments, we suspected that selective killing of a certain type of cells played an important role. As observed by one of the authors (SADO 1959), the secondary spermatogonia of the silkworm are completely destroyed by acute X-ray doses of more than 500r, while the primary spermatogonia are fairly resistant. We may assume a higher mutability of those highly sensitive cells. Thus, by acute irradiation, the secondary spermatogonia probably were killed completely, leaving only primary spermatogonia with lower mutation rates for transmission to the offspring. Under chronic irradiation, on the other hand, both types of cells could have survived, and the sensitive secondary spermatogonia may have been responsible for the higher mutation rates.

The relative proportion of the primary to the secondary spermatogonia is known to decrease with the development of the insects (Figure 1). This consideration suggested a further test.

*The third experiment (Expt. 602):* Chronic exposure was given to two groups, one earlier and one later, dividing the period in question into two halves. Because of the limited length of the period, irradiation of the early group extended into earlier stages of embryonic development by two days, while that in the later group extended into the third instar by one and a half days (Figure 1). For acute exposure two experimental groups were set up. In the acute-γ 1 group irradiation was given soon after hatching while in another group, acute-γ 2, the larvae were irradiated on the first day of the third instar, when the period of irradiation terminated in the chronic-γ 2 group. The results are shown in Table 3.

It will be noted from the table that the mutation rates are higher in Expt. 602 than those obtained in the preceding experiments.

The most striking fact is that unexpectedly high rates were obtained in the

acute-γ 1 group both for spermatogonia and oögonia. The irradiated cells are supposed to have been at the stage of primordial germ cells.

Secondly, consistent results with those of RUSSELL *et al.* are obtained if comparisons are made between two corresponding groups, acute-γ 1 and chronic-γ 1. That is, the mutation rates, both for spermatogonia and oögonia, in the acute group are approximately twice as high as those in the chronic group.

Thirdly, the situation is reversed for both spermatogonia and oögonia irradiated at later stages; here mutation rates in the acute group were only approximately half those in the chronic group.

*The fourth experiment (Expt. 603):* To check the high mutation rates observed in the acute 1 and the chronic 2 groups of Expt. 602 the fourth experiment was carried out. In the acutely irradiated groups, four ages of irradiation were chosen that allowed a comparison of induced mutation rates at successive stages of the spermatogonial or oögonial development: i.e., irradiation three days before hatching, soon after hatching, during the late first instar, and during the late second instar. In the chronically irradiated groups, the experimental design was almost the same as in Expt. 602 except that in both groups the period of irradiation was shifted by one day toward the earlier stages.

The results are given in Table 4. They are completely consistent with those obtained in the foregoing three experiments. Among the acutely irradiated groups, mutation rates were again highest at the stage of hatching, decreasing gradually toward both earlier and later stages. It reached a minimum around the second molt of the larvae. The two chronically irradiated groups had similar high mutation rates with a slight increase in the later group.

When comparisons are made between the corresponding groups of chronic

TABLE 3

*Results of Expt. 602*

| | Total no. observed | Mut. detd. | *pe* Freq. | Induced rate/r | Mut. detd. | *re* Freq. | Induced rate/r | Confidence interval for freq. *pe* | *re* |
|---|---|---|---|---|---|---|---|---|---|
| | | | $\times 10^{-5}$ | $\times 10^{-7}$ | | $\times 10^{-5}$ | $\times 10^{-7}$ | | |
| a. Irradiation of spermatogonia *pe re* ♀ × ++ ♂ | | | | | | | | | |
| Acute-γ 1 | 69,700 | 108 | 154.9 | 13.80 | 61 | 87.5 | 8.66 | 127.1–187.1 | 66.9–112.4 |
| Acute-γ 2 | 54,283 | 35 | 64.5 | 4.76 | 2 | 3.7 | 0.28 | 44.9– 89.7 | 0.4– 13.3 |
| Chronic-γ 1 | 70,535 | 75 | 106.3 | 8.94 | 27 | 38.3 | 3.74 | 83.6–133.3 | 25.2– 55.7 |
| Chronic-γ 2 | 81,026 | 102 | 125.9 | 10.90 | 43 | 53.1 | 5.22 | 102.6–152.8 | 38.4– 71.5 |
| b. Irradiation of oögonia ++ ♀ × *pe re* ♂ | | | | | | | | | |
| Acute-γ 1 | 173,147 | 203 | 117.2 | 11.16 | 163 | 94.1 | 9.01 | 101.7–134.5 | 80.2–109.8 |
| Acute-γ 2 | 118,185 | 36 | 30.5 | 2.49 | 26 | 22.0 | 1.80 | 21.3– 42.2 | 14.4– 32.2 |
| Chronic-γ 1 | 196,917 | 111 | 56.4 | 5.05 | 88 | 44.7 | 4.07 | 46.5– 62.8 | 35.8– 55.1 |
| Chronic-γ 2 | 206,585 | 123 | 59.5 | 5.39 | 56 | 27.1 | 2.31 | 49.5– 71.0 | 20.5– 35.2 |

and acute irradiation, i.e., between chronic 1 and the average of acute 1 and 2; and between chronic 2 and the average of acute 3 and 4 (Table 5), one may again notice two reciprocal types of dose-rate dependence of mutation frequency: one type shows a higher mutation rate in the acutely irradiated groups than in the chronically irradiated groups, and the other shows the reverse reaction. The former is observed when the germ cells were irradiated during the earlier half of the tested period, although for spermatogonia the difference in mutation rates between the acute and the chronic groups is small. The latter is clearly seen when irradiations were administered during the later half of the period.

TABLE 4

*Results of Expt. 603*

| | Total no. observed | Mut. detd. | *pe* Freq. | Induced rate/r | Mut. detd. | *re* Freq. | Induced rate/r | Confidence interval for freq. *pe* | *re* |
|---|---|---|---|---|---|---|---|---|---|
| a. Irradiation of spermatogonia *pe re* ♀ × + + ♂ | | | | | | | | | |
| | | | $\times 10^{-5}$ | $\times 10^{-7}$ | | $\times 10^{-5}$ | $\times 10^{-7}$ | | |
| Acute 1 | 150,627 | 131 | 87.2 | 7.9 | 72 | 47.8 | 4.6 | 72.7–103.2 | 37.4– 60.2 |
| Acute 2 | 150,181 | 217 | 144.5 | 13.6 | 99 | 64.6 | 6.3 | 126.6–165.1 | 53.6– 80.2 |
| Acute 3 | 164,020 | 160 | 97.5 | 8.9 | 75 | 45.7 | 4.4 | 83.0–113.9 | 36.0– 57.3 |
| Acute 4 | 164,484 | 63 | 38.3 | 3.0 | 26 | 15.8 | 1.4 | 31.4– 47.1 | 10.3– 23.2 |
| Control | 157,987 | 13 | 8.2 | ... | 3 | 2.0 | ... | 4.4– 14.1 | 0.2– 4.6 |
| Chronic 1 | 185,739 | 187 | 100.7 | 9.3 | 114 | 61.4 | 5.9 | 86.8–116.2 | 50.7– 73.7 |
| Chronic 2 | 172,724 | 212 | 122.7 | 11.5 | 110 | 63.7 | 6.2 | 106.8–140.4 | 52.3– 76.8 |
| b. Irradiation of oögonia + + ♀ × *pe re* ♂ | | | | | | | | | |
| Acute 1 | 235,784 | 146 | 61.9 | 5.3 | 129 | 54.7 | 4.5 | 52.3– 72.8 | 45.8– 65.0 |
| Acute 2 | 262,595 | 240 | 91.4 | 8.2 | 215 | 81.9 | 7.2 | 82.2–103.7 | 71.3– 93.6 |
| Acute 3 | 279,510 | 165 | 59.0 | 5.0 | 146 | 52.2 | 4.3 | 50.4– 68.8 | 44.1– 61.4 |
| Acute 4 | 281,861 | 57 | 20.2 | 1.1 | 29 | 10.3 | 0.1 | 15.3– 26.2 | 6.9– 14.8 |
| Control | 293,249 | 27 | 9.2 | ... | 28 | 9.5 | ... | 6.1– 14.4 | 6.3– 13.8 |
| Chronic 1 | 342,839 | 183 | 53.4 | 4.4 | 138 | 40.3 | 3.1 | 45.9– 61.7 | 33.7– 47.6 |
| Chronic 2 | 263,169 | 159 | 60.4 | 5.1 | 114 | 43.3 | 3.4 | 51.4– 70.6 | 35.7– 52.0 |

TABLE 5

*Comparisons of induced mutation rates between the corresponding groups of acute and chronic irradiation of spermatogonia and oögonia (Mutation rates are shown per r per locus)*

| Type of irradiated cells | Locus | Earlier half: Acute Avg. 1&2 | Earlier half: Chronic 1 | Later half: Acute Avg. 3&4 | Later half: Chronic 2 |
|---|---|---|---|---|---|
| | | $\times 10^{-7}$ | $\times 10^{-7}$ | $\times 10^{-7}$ | $\times 10^{-7}$ |
| Spermatogonia | *pe* | 10.8 | 9.3 | 6.7 | 11.5 |
| | *re* | 5.5 | 5.9 | 2.9 | 6.2 |
| Oögonia | *pe* | 6.8 | 4.4 | 3.0 | 5.1 |
| | *re* | 5.9 | 3.1 | 2.2 | 3.4 |

## DISCUSSION

A summary of the results of Experiments 594, 601, 602, and 603 is shown graphically in Figures 2 and 3. It is noteworthy that in the acute irradiation group the mutation frequency has a rather sharp maximum around the stage of hatching and decreases with the advancement of development, reaching a minimum in the late second instar, while in the chronic irradiation group there is a slight increase in mutation frequencies in the later half of the tested period compared with those in the earlier half. This finding suggests that induced mutation rate is higher for the secondary spermatogonia, if they survive, than for primary spermatogonia.

Comparison of mutation frequencies between the acute and chronic groups is given in Table 6. For simplicity's sake, the induced mutation frequencies of the acute groups have been calculated by averaging the changes in mutation frequencies over the corresponding period by use of the smoothed frequency curves given in Figures 2 and 3; the frequencies of the chronic groups have been obtained from the results of Expt. 603 combined with those of Expt. 602; and spontaneous mutation frequencies have been subtracted from the experimental frequencies of the irradiated groups. Though a more detailed statistical treatment of the data will be given elsewhere, it may be clear from Table 6 that there are two reciprocal types of dose-rate dependence of induced mutation rate.

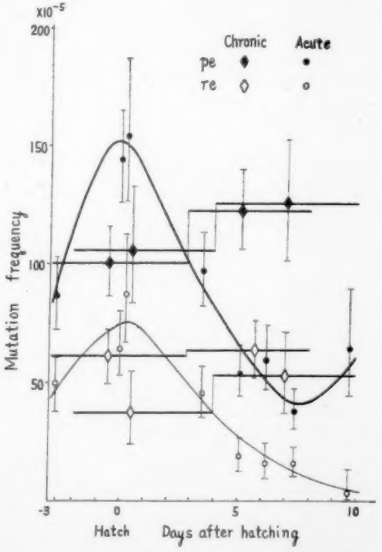


FIGURE 2.—Changes in mutation frequencies with the development of the silkworm for acute and chronic irradiation of spermatogonia.

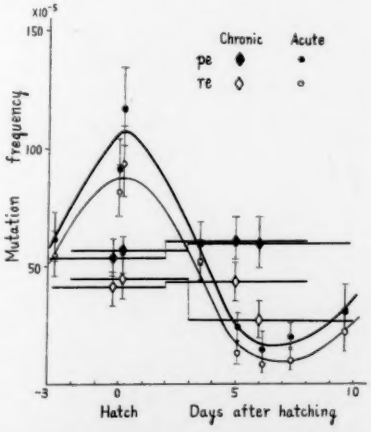


FIGURE 3.—Changes in mutation frequencies with the development of the silkworm for acute and chronic irradiation of oögonia.

a) A type of dose-rate dependence consistent with findings of RUSSELL *et al.*, i.e., lower mutagenic effectiveness at chronic than at acute irradiations, was found in the silkworm only in very early stages of spermatogonia as well as of oögonia, when primordial germ cells are the prevalent cell type in the testis (Type I). b) A different type of dose-rate dependence, i.e., higher mutagenic effectiveness of chronic than acute irradiations was found in later stages of spermatogonial and oögonial development (Type II).

For an explanation of these apparently contradictory findings, the following provisional assumptions are made.

1. *Primary mutation rates differ according to the stage of germ cells:* They may be high at the stage of primordial germ cells, low at the primary spermatogonial stage and intermediate at the secondary spermatogonial stage. Marked changes in radiation induced mutation rates according to the stages of gametogenesis have already been revealed in the silkworm (TAZIMA 1958, 1959) as well as in Drosophila and mouse (LÜNING 1952; AUERBACH 1954; BATEMAN 1956, 1958; and MULLER 1958). However, differences in induced mutation rates between very early gonial cell stages have not been previously observed.

2. *Repair of primary mutations:* A hypothesis was proposed by RUSSELL, RUSSELL and KELLY (1958), when they found much lower mutagenicity of chronic irradiation than acute irradiation of young germ cells of the mouse. They assumed that there are two kinds of mutation, reparable and irreparable, and repair of reparable mutations occurs at low radiation intensity level, but it is less probable at high radiation intensity. This interpretation fitted in quite well with their results. The same may explain the Type I dose-rate dependence in our present experiments. However the hypothesis can not explain the Type II

TABLE 6

*Comparisons of induced mutation frequencies between the acute and chronic irradiation of silkworm spermatogonia and oögonia (per 1000r per locus)*

| Stage irradiated | Sex | Locus | Radiation-induced mutation frequency | | Ratio of acute to chronic |
|---|---|---|---|---|---|
| | | | Acute group | Chronic group | |
| | | | $\times 10^{-5}$ | $\times 10^{-5}$ | |
| Earlier half* | Male | *pe* | 108.6 | 85.9 | 1.26 |
| | | *re* | 61.0 | 53.8 | 1.14 |
| | Female | *pe* | 78.4 | 47.6 | 1.65 |
| | | *re* | 66.8 | 35.3 | 1.89 |
| Later half† | Male | *pe* | 39.5 | 107.4 | 0.37 |
| | | *re* | 17.3 | 59.1 | 0.29 |
| | Female | *pe* | 19.3 | 63.1 | 0.31 |
| | | *re* | 12.1 | 31.2 | 0.39 |

* The period from three days before to 3.5 days after hatching.
† The period from 3.5 days through ten days after hatching.

dose-rate dependence that occurs in later stages of spermatogonial development, because the acute irradiation resulted in lower mutation frequency than the chronic one.

No evidence is available at present that suggests whether or not a repair process does operate in later stages. But it is conceivable that after chronic irradiation, repair of reparable primary mutations may take place in germ cells with a long mitotic cycle but might be less frequent in those with a short mitotic cycle. Primary and secondary spermatogonia are supposed to have a much shorter cell cycle than the primordial germ cells. Hence, repair is supposed to play an important role only in the primordial germ cells under such circumstances. The estimated length of cell cycle for each type of cell will be reported elsewhere.

3. *Selective killing of a specific type of cell:* The Type II dose-rate dependence found in the present experiment may be interpreted by a hypothesis of selective killing. Among three types of cells, the secondary spermatogonia, which presumably correspond to type B spermatogonia of the mouse (OAKBERG 1955) are extraordinarily sensitive to radiation. They are selectively killed by intense dose rates, while primordial germ cells and primary spermatogonia can survive under acute dose-rate conditions such as were used in our experiments (SADO 1959). Under chronic irradiation all three types of cell can survive and are assumed to be transmitted to the next generation. This has been shown cytologically in the rat (CASARETT and CASARETT 1957). The lower mutagenicity of acute irradiation in later stages may, therefore, be attributed to the marked decrease in the number of secondary spermatogonia of relatively high mutation rate. Accordingly, observed mutation frequency in acute groups reflects only that of primary spermatogonia of low mutation rate.

For the mouse, RUSSELL (1956) has suggested selective killing in explanation of his finding that acute irradiation with 1000r produced fewer mutations than 600r. This was confirmed by the fact that fractionation of 1000r acute radiation raised mutation frequency to approximately the value expected from a straight-line dose effect curve for acute radiation (RUSSELL *et al.* 1959).

If the hypothesis proves true, the same principle may operate through all stages of development wherever cell types are heterogeneous, and the phenomenon will be marked when excessively radiation sensitive cells such as secondary spermatogonia are involved. This will explain why type II dose-rate dependence was observed in later stages but not in early stages.

In the above arguments, we have limited ourselves to the discussion only of male germ cells, because cytological evidence has so far been obtained only for the male. Though cytologically not yet supported, the same explanation may apply also to the female germ cells.

With these assumptions, both types of dose-rate dependence can be explained without contradiction.

## SUMMARY

Dose-rate dependence of radiation-induced mutation rate was studied in silkworm spermatogonia and oögonia by the use of the specific locus method.

Both sexes of a wild-type strain were exposed to X-rays or $Cs^{137}$ $\gamma$-rays of high dose rate or to $Co^{60}$ $\gamma$-rays of low dose rate at a definite stage or during a definite period in early larval stages. The dose-rate ratios between the acute and chronic irradiations were 2500:1–7600:1. The total doses given were 950r or 1000r.

Two types of dose-rate dependence of radiation-induced mutation rate were found.

In one type mutagenic effectiveness of chronic irradiation is lower than that of acute irradiation (Type I), and in the other mutagenic effectiveness is higher in chronic irradiation than acute irradiation (Type II). The former is observed only in the very young larval stage when the primordial germ cells are prevalent in the testis, while the latter is found when later stages of spermatogonia or oögonia are irradiated.

For Type I dose-rate dependence, repair of primary mutations seems to be responsible, whereas for Type II dose-rate dependence selective killing of a specific type of cells by intense exposure seems to play an important role. The relationship between our finding and that of RUSSELL *et al.* (1958, 1959) is discussed.

Radiation-induced mutation frequency is remarkably high at the very early stage of larva, i.e., around the time of hatching when the primordial germ cells are prevalent in the gonads.

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# AN EVALUATION OF THE MULTIPLE CROSSOVER AND SIDE-CHAIN HYPOTHESES BASED ON AN ANALYSIS OF ALPHA DERIVATIVES FROM THE $A^b$-P COMPLEXES IN MAIZE[1]

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VARIOUS gene complexes which behave as alleles of $A_1$ in maize, and which carry the general designation $A^b$, give rise infrequently, but consistently, to a characteristic mutant form, designated $A^a$ or alpha ($\alpha$), with intermediate effects on plant and aleurone phenotype. The occurrence of this mutant form is associated with one of the two types of recombinants for spanning marker loci with a far higher frequency than anticipated on the basis of coincidental exchange in the marked segment (LAUGHNAN 1949, 1952a, 1955a). It was concluded that alpha is itself one element of a linked complex that also includes a member, designated beta ($\beta$), whose phenotypic effect on plant and aleurone is full purple and is, on most criteria, indistinguishable from the total effect of the $A^b$ complex itself. The isolation (LAUGHNAN 1956, 1961) of the beta element from the various $A^b$ complexes in strands whose recombinant markers are complementary to those carrying the alpha isolate confirms both the interpretation of alpha and beta as closely linked but separable elements, and the assignment of sequences to the members.

However, as it stands, the scheme described above does not provide an obvious explanation for the infrequent but regular occurrence, from these same $A^b$ complexes, of alpha-carrying strands that are nonrecombinants for the marker loci (LAUGHNAN 1949, 1952a, 1955a, c, 1961). The purpose of this report is to consider, for their bearing on the origin of the nonrecombinant alpha cases, certain specific mechanisms which have been proposed to account for the occurrence of somewhat similar exceptions in Neurospora (GILES 1951; MITCHELL 1955; ST. LAWRENCE 1956; FREESE 1957; CASE and GILES 1958; STADLER 1959), in Aspergillus (PRITCHARD 1955, 1960a, b; CALEF 1957), in yeast (ROMAN and JACOB 1958), in bacteriophage (STREISINGER and FRANKLIN 1956; CHASE and DOERMANN 1958), in maize (STADLER and NUFFER 1953; EMMERLING 1958), and in Drosophila (DEMEREC 1926, 1928; CHOVNICK 1958).

In most of these studies single-strand analysis of wild-type recombinants from heterozygotes involving two closely linked, functionally related mutants gave consistently anomalous distributions for the outside marker combinations. Data (Table 1) on meiotic recombination between two cysteineless mutants (STADLER 1959) in Neurospora are typical of inconsistencies encountered in similar in-

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TABLE 1

*Distribution of marker combinations among cysteine-independent spores from the* cys-c/cys-t *heterozygote**

| Cross | Selection | | Strand types with respect to marker loci: Recombinants | | Nonrecombinants | |
|---|---|---|---|---|---|---|
| | | | *un ylo* | + + | *un* + | + *ylo* |
| *un cys-c* + ** / + *cys-t ylo* | Cysteine independent | No. | 18 | 25 | 68 | 26 |
| | | Percent | 13.1 | 18.3 | 49.6 | 19.0 |

* This table is adapted from STADLER 1959.
** Map distances established by conventional crossover analysis of progeny from this cross: *un* 2.1 *cys* 5.6 *ylo*.

vestigations of other loci. If, for example, it is held that the selected cysteine independent arises by some mechanism not involving the participation of both cysteineless cistrons, an argument which is at odds with the observation that self (within strain or homoallelic) crosses yield prototrophs with extreme rarity, or not at all, we find that among prototrophs the frequency (31.4 percent) of strands that are recombinant for the outside markers is far in excess of the predicted value (7.7 percent) for coincidental exchange based on the genetic map length of the *un–ylo* segment. On the other hand, the argument that the *cys* independent results from conventional exchange between homologues within the *cys* cistron, which involves the assumption that *cys-c* and *cys-t* occupy different sites, is at variance with the high frequency of strands that are nonrecombinant for the outside markers, and with the near equality of the two recombinant classes. In this and in other studies yielding similar contradictory results, it is evident that the event giving rise to nonmutant progeny has neither the characteristics of gene mutation nor of conventional crossing over but might be considered to have attributes of both.

The various schemes proposed to explain these unusual results have, in addition, had to take account of the unexpected occurrence of aberrant asci in tetrad analyses of individuals heterozygous for closely linked loci. Thus, again using the studies on the cysteine mutants (STADLER 1959) as an example, tetrad analysis of the *cys-c/cys-t* trans heterozygote gave three asci, of the 153 analyzed, that carried a cysteine independent spore pair. Backcross tests of the nine cysteineless strains from these asci established that the *cys-c cys-t* double mutant, expected if the recombinational event giving rise to the wild type were reciprocal, was not represented. Similar anomalous results, featuring the absence of the complementary recombinant type, have been obtained at other loci in Neurospora (MITCHELL 1955; CASE and GILES 1958), in yeast (LEUPOLD 1958; ROMAN and JACOB 1958), and in Aspergillus (STRICKLAND 1958). It should be emphasized, however, that recombinations yielding the wild type are not in all cases nonreciprocal; tetrad analyses involving pantothenic acid mutants (CASE and GILES 1958) reveal that reciprocal recombination also occurs, and in Aspergillus, where STRICKLAND (1958) analyzed a number of loci for this phenomenon, only rare instances of nonreciprocal recombination were encountered.

*The hypotheses*

Two basic schemes have been proposed to account for the occurrence, as a nonreciprocal recombinant, of the wild-type revertant from the type $m_1/m_2$ heterozygote, and for the unexpected distributions of outside markers among such recombinants. These models are treated here in some detail so that we may consider, in the sections that follow, whether they represent valid explanations for the anomalous alpha derivatives in maize.

*Single mechanism:* According to this scheme both reversion to independence, and recombination for outside markers, are ascribable to the same basic phenomenon. On the basis of extensive analyses of mitotic and meiotic recombinants within the *ad8* cistron in Aspergillus, PRITCHARD (1955, 1960a,b) has proposed that recombination takes place in localized regions of effective pairing. The evidence he presents for a linear separation of the various mutant forms in this cistron is convincing. Assuming that the adenine independents which are selected in his experiments result from crossing over between mutational sites in various trans heterozygotes (e.g. *ad8/ad11*) the excessive recombination for outside markers among these revertants (negative interference) is explained on the basis of multiple exchanges within a short, effectively paired region which spans the *ad* cistron. On the hypothesis of localized, effective pairing, the apparent high negative chromosome interference observed among the selected revertants does not imply that one crossover enhances the probability of another in its neighborhood but rather follows logically from the assumption that (a) effective pairing in the vicinity of the adenine locus occurs in only a small fraction of the cell population sampled, and (b) when pairing is realized, the probability of exchange within the paired region is sufficiently high (PRITCHARD estimates 0.6) to account easily for the occurrence of multiple crossover strands.

On a copy-choice model the author (PRITCHARD 1960a) proposes that duplication of the two new strands progresses simultaneously and that switches in copy from one template to the other, which may occur only within the paired segment, are highly correlated in Aspergillus, thus accounting for the regular occurrence there of reciprocal recombinants. PRITCHARD's suggestion that nonreciprocal recombination, as encountered notably in Neurospora and yeast, may be attributed to slight displacement of the point of switching of one duplicating strand with respect to the other, is not unlike the explanation offered by FREESE (1957) who also postulates regions of localized, intimate pairing within which multiple switches may occur at the time of duplication; but on the latter scheme the two duplicating strands need not have equal numbers of switch points within the pairing region.

Since maize does not offer the opportunity of tetrad analysis, it is not possible to say whether the exceptional alpha occurrences are reciprocal, nonreciprocal or both. The essential feature of the schemes based on a single mechanism, so far as they apply to the case in maize, is that multiple exchanges within relatively short (PRITCHARD estimates a mean length of pairing region of 0.4 of a conventional map unit), effectively paired segments, whether by copy-choice or by exchanges between previously duplicated strands, are responsible for both the

reversion event and the excessive recombination for outside markers among the selected revertants. Specifically, on this hypothesis, the alpha isolations from $A^b$ compounds in maize are all attributable to crossing over; accordingly, those that are nonrecombinants for markers must represent isolations on strands that have been involved in an even number of exchanges in the marked segment.

*Separate mechanisms:* The various schemes that fall in this category assume that reversion at the locus under investigation, and the excessive recombination for outside markers noted among the selected revertants, are due to different mechanisms. However, since there is a high correlation between reversion and recombination for outside markers, it must be assumed that both events are favored by a third condition which is satisfied in some, but not all, individuals of the population under investigation.

Perhaps the most appealing basis on which to argue separate events is to consign them to different spatial realms. Thus, ROMAN and JACOB (1958) favor the concept of a branched chromosome to explain the correlation between reversion at the isoleucine locus and recombination between the histidine-1 and tryptophane-2 marker loci in yeast, and in particular to account for the differential effect of ultraviolet radiation on these two processes. According to this model, in the trans heterozygote involving two isoleucine mutants designated $i_a$ and $i_b$, the latter are considered to represent defects at different sites in a DNA side chain (cistron). The selected isoleucine-independent revertant results from recombination between the differently-defective side chains to produce one that is functionally normal, though it is clear that this event itself must be nonreciprocal to explain the observation that the complementary double-mutant type does not occur simultaneously. The abnormally high rate of recombination for outside markers among the revertants is explained by assuming that both the hypothetical side-chain event and conventional crossing over in the protein backbone are favored when some third condition, such as intimate pairing of homologues in the isoleucine region, obtains. On this scheme, ultraviolet radiation is held to affect differentially the side-chain and backbone events, though the authors agree that the results are not inconsistent with a scheme, such as that proposed by FREESE (1957), involving a single mechanism without the assumption of side chains.

In testing the fit of the maize results to predictions based on this hypothesis it should be emphasized that this scheme does not provide for a linear separation of closely linked, mutant sites along the backbone of the chromosome and hence prohibits the occurrence of a revertant as a result of a conventional crossover. Therefore, neither of the recombinant marker classes should be favored on this account though they may still be disproportionate if the two kinds of side-chain reversions have different frequencies and if the frequencies of coincidental crossovers in the marked segments to left and right are different.

Because it may have special significance for the anomalous derivatives in maize, a variation of the conservative side-chain hypothesis presented above should be considered. According to this modification the closely linked, mutant sites reside in separate but adjacent side chains. STADLER (1959) has proposed

and favors such a model to explain the results obtained from the analysis of the cysteineless mutants; thus, in the case of the *cys-c/cys-t* heterozygote, one homologue carries an impaired *cys-c* side chain and an adjacent side chain that is normal for the *cys-t* impairment, while the other homologue carries the complementary condition. Although this model suffers the disadvantage of spatial discontinuity within the cistron, it allows for the occurrence of reversion either as a result of a crossover in the backbone or as a result of side-chain events. According to this scheme the alpha and beta members of $A^b$ complexes in maize are considered to reside in different but adjacent side chains.

In her original report on the pyridoxine mutants, MITCHELL (1955) proposed that the occurrence, in asci from trans *pdx/pdxp* heterozyogtes, of occasional pyridoxine independent spore pairs unaccompanied by the reciprocal double-mutant type might be attributed to gene conversion (WINKLER 1930) leading to the recovery, in a single duplicating strand, of the normal counterparts of both mutant forms, for which she did not assume linear separation. Since gene conversion itself will not account for the excessive recombination of outside markers encountered among the revertants in single strand analyses, it was assumed that both gene conversion and crossing over are favored by a particular circumstance, held to prevail in some cells of the population but not in others, such as intimate pairing between homologues in the neighborhood of the pyridoxine locus. Unfortunately, the term "gene conversion" has come into widespread use to identify, in a general way, those recombinational phenomena which, though they are correlated with exchanges between outside markers, can not be the product of conventional crossing over alone. But considered from an operational standpoint, "gene conversion" has usually been defined loosely or not at all, and has come into use to describe results, in much the same way that "position effect" may be used to describe a phenomenon, but not to explain it. In the writer's opinion, if the term "gene conversion" has been loosely defined, or misapplied, the fault lies mainly in the lack of a convincing conceptual basis for the phenomenon. Gene conversion is held to mean (WINKLER 1930) that alleles in heterozygotes are able to convert *inter se* their counterparts in homologues, thus to explain the occasional exceptional ratios encountered in asci (LINDEGREN 1949, 1953, 1955). But the most reasonable basis for the occurrence of such an event is by partial or complete substitution of the genetic material of one allele by the other occurring as a result either of an aberrant duplication or of exchanges, which are not exactly reciprocal, between already duplicated strands. Thus, if it were established beyond doubt that multiple exchanges within short segments of a chromosome actually do occur, and that they may on occasion be nonreciprocal, the presumed cases of conversion would be credited to this phenomenon if only because a mechanism for gene conversion, as precise as that for multiple exchange, has not been formulated. It is not surprising, therefore, that those who have used the term "gene conversion" to describe superficially the results they encounter have in many cases proposed one or another more specific recombinational mechanism to explain them. In any case, as will be evident later, in the interpretation of the maize experiments it is impossible to distinguish operationally between gene

conversion and the more clearly defined recombinational mechanisms discussed above and for obvious reasons the writer prefers to deal with the latter types of hypothetical models.

### *The* $A^b$ *complexes in maize*

Three $A^b$ complexes of separate origin have been the objects of intensive genetic analyses. One of these, here designated $A^b$-Ec, is the original $A^b$ of Ecuador extraction whose unusual phenotypic effects were described by EMERSON and ANDERSON (1932). The other two, having the general designation $A^b$-P, and referred to specifically as $A^b$-Lima and $A^b$-Cusco, were originally isolated from Peruvian accessions; they have a similar basic structure though they exhibit slight differences in action which indicate that they are not identical.

It has been established (LAUGHNAN 1952a, 1955a) that both the $A^b$-Ec and $A^b$-P forms are complexes consisting of separable alpha and beta elements, but it is also evident, from previous investigations, that these complexes exhibit rather remarkable differences which may be summarized as follows:

(a) The alpha element of the $A^b$-P complexes is decidedly weaker in its effect on plant and aleurone pigmentation than that of $A^b$-Ec.

(b) Crossover analysis reveals that the sequence of alpha and beta elements in the $A^b$-P complexes is the reverse of that in $A^b$-Ec; thus, using the symbol C to designate the centromere, the order of members in the $A^b$-Ec complex is C:$\alpha$:$\beta$ whereas the sequence of members in the $A^b$-P complexes is C:$\beta$:$\alpha$.

(c) Whereas the $A^b$-Ec and $A^b$-P complexes yield alpha derivatives that are recombinants for marker loci with similar frequencies (*ca.* $3 \times 10^{-4}$), there is a tenfold difference in the frequency of occurrence of the nonrecombinant alpha derivatives from the $A^b$-Ec complex (*ca.* $5 \times 10^{-5}$) as compared with that from the $A^b$-P complexes (*ca.* $5 \times 10^{-4}$).

Analyses (LAUGHNAN 1952b, 1955b) of the derivatives from homozygous $A^b$-Ec individuals, and from certain heterozygotes involving $A^b$-Ec, indicate that alpha and beta, or the segments in which they reside, are members of an adjacent duplication in which the genetic materials are ordered in the same sequence; it is evident that $A^b$-Ec, like Bar in *Drosophila melanogaster*, is a tandem, serial duplication whose members engage in oblique synapsis with homologous elements of the homologue. Moreover, similar evidence (LAUGHNAN 1955a, 1961) from $A^b$-P homozygotes establishes that the beta- and alpha-carrying segments of this complex engage in oblique synapsis, indicating that they too are members of a tandem, serial duplication. Finally, the occurrence of viable alpha derivatives from $A^b$-Ec/$A^b$-P heterozygotes (LAUGHNAN 1955a) establishes that these complexes are not simply gross inversions with respect to each other, but rather that they have their genetic material ordered in the same sequence but with the alpha and beta segments interchanged as represented below (the numbers here have the significance only of indicating sequence of genetic material):

| $A^b$-Ec | | | | | | $A^b$-P | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $\alpha$ | | | $\beta$ | | | $\beta$ | | | $\alpha$ | | |
| 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |

On the basis of these models for the two complexes there is no difficulty in accounting for the occurrence of the recombinant alpha (pale) derivative; since alpha is the left-most element of the $A^b$-Ec complex it is isolable on a strand that is recombinant for the distal marker locus, and conversely, crossover isolation of alpha from the $A^b$-P complex is associated with recombination for the proximal marker. The pale phenotype of both crossover isolates is thus assignable to loss, to the complementary crossover strand, of the purple-acting beta element which masks the alpha effect when they are associated in the complex.

But the isolation of alpha (or loss of beta effect) from these complexes as a nonrecombinant for the marker loci does not follow from simple inspection of the model presented above and therefore requires special consideration. One of the mechanisms proposed to account for the occurrence of the nonrecombinant alpha derivatives is gene mutation of the beta member to a null level. Such a qualitative change in the beta member, rendering it impotent in the production of pigment, would lead directly to the expression of the adjacent alpha member; and since, on this hypothesis, the homologue does not participate in the mutational event, the alpha occurrence, except for randomly distributed, coincidental exchanges, would not be associated with recombination for marker loci.

This possibility has been explored experimentally using three separate approaches:

(a) Beta elements, isolated by crossing over from the alpha member of $A^b$-P complexes, were subjected to mutational analysis in heterozygotes with the complex from which they were derived, to analyze for the hypothetical beta mutational event (LAUGHNAN 1957, 1961).

(b) The frequency of occurrence of the nonrecombinant alpha was investigated in a synthetic complex of $\alpha$:$A$ constitution in which the $A$ member was substituted for beta in the $\alpha$:$\beta$ complex of $A^b$-Ec (LAUGHNAN 1961).

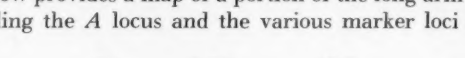
(c) Nonrecombinant alpha derivatives, along with corresponding crossover isolates from both complexes, were themselves subjected to crossover analyses in attempts to isolate the hypothetical null form of beta which is presumed, on the gene mutation hypothesis, to be present in the alpha of nonrecombinant origin (SARMA 1959).

These investigations indicate (LAUGHNAN 1955c, 1961) that mutation of the beta member of the $A^b$ complexes to a null form, if it occurs at all, is so infrequent that it can not be the mechanism responsible for the occurrence of the vast majority of nonrecombinant alpha derivatives.

In considering the possibility that the models discussed in a foregoing section might account for the origin of the anomalous alpha derivatives in maize, it should be emphasized that the typical system employed in the pertinent studies with microorganisms involves a trans type heterozygote in which each of the homologues carries a different mutant or defective site within a specified functional unit or cistron. Characteristically, each of these mutant strains and their heterozygote are unable to grow in the absence of a specific metabolite, whose synthesis proceeds normally in the presence of an unimpaired cistron. But among the progeny of the heterozygote produced in mitotic or meiotic cycles, as the

case may be, there are rare occurrences of revertants that no longer require the metabolite. These revertants are the highly selected sample of the population which is the object of further genetic analyses. Since the individual mutant strains themselves show only negligible rates of reversion, the selection of revertants from the heterozygote appears to enforce the participation of both homologues in the reversion event. On the other hand, the $A^b$ complexes in maize carry the alpha and beta sites in a single homologue in what, by analogy, might be considered the cis phase; in pursuing the analysis of derivatives from such a complex, attention is focused on the isolation of the alpha member, an event which, unlike the reversion dealt with in the microorganisms, need not necessarily involve the homologue.

The figure below provides a map of a portion of the long arm of chromosome 3 in maize, including the *A* locus and the various marker loci employed in the




experiments to be discussed. The symbol *T* refers to translocation 2-3d (ANDERSON and BRINK 1940) which has been used extensively to mark a segment proximal to the *A* locus; it refers specifically to a $2^3$ chromosome carrying the *A* locus in the interchanged portion of chromosome 3. In translocation heterozygotes, the symbol *N* will refer to a noninterchanged chromosome 3. Since marked heterozygotes to be analyzed for alpha occurrences were routinely crossed with homozygous testers not carrying the translocation, the presence or absence of the interchanged chromosome in exceptional offspring was easily determined by the presence or absence of 50 percent aborted pollen, the former of which is typically associated with plants that are heterozygous for the translocation; and since ears of plants heterozygous for this translocation regularly exhibit abortion of half of their ovules, the pollen classification referred to above was routinely confirmed by scoring the ears of exceptional individuals for the aborted condition. The *sh*$_2$ (shrunken-2) factor, which in homozygous recessive condition effects a drastic collapse of the mature endosperm, is distal to *A* and marks a segment whose genetic length is only one-quarter unit. Because of its close proximity to *A*, *sh*$_2$ has been preferred as a marker to the recessive factor *et* (etched endosperm, virescent seedling), but as we shall see, the latter may be used to advantage in certain instances. It should be observed that the genetic lengths of the *T* and *sh* marked segments on either side of the *A* locus, 7.1 and 0.25 units, respectively, are almost exactly the same as the corresponding segments, 6.0 and 0.2, on either side of the *ad8* cistron, defined by the markers *bi* (biotinless) and *y* (yellow), in PRITCHARD's (1960b) studies with Aspergillus.

In considering the results of investigations of the $A^b$ complexes for their fit to the models discussed in a foregoing section we shall, for the sake of convenience, use the term *multiple exchange* in referring to the scheme that attributes both the reversion (primary event) and the excessive recombination for outside markers (secondary event) to a single mechanism, that is, to crossing over. The

alternative model, according to which the primary and secondary events are based on separate mechanisms, will be referred to as the *side-chain* hypothesis. In pursuing the analysis we may anticipate obtaining the answers to two different but not unrelated questions: (a) is it possible to explain the occurrence of both the recombinant and nonrecombinant alpha isolates according to one, both, or neither of these two hypotheses, and (b) if neither scheme represents a plausible explanation for the alpha occurrences, to what extent do the available data bear on whether these mechanisms may nevertheless operate in maize?

### *Evidence from deficiency heterozygotes*

According to the multiple-exchange hypothesis, the alpha component of the marked heterozygote carrying the beta:alpha complex in one homologue and recessive *a* (associated with colorless phenotype) in the other, may be isolated as an apparent nonrecombinant for the marker loci in one of two ways, both of which involve double crossover strands (Figures 1B, 1C). One of these carries the parental markers of the $A^b$-P chromosome while the reciprocal event leads to an alpha strand carrying the parental markers of the homologue. But in either case one of the exchanges, hereafter referred to as the primary event, must occur within the complex, that is, between the beta and alpha members. If both the recombinant and nonrecombinant alpha derivatives are dependent on a crossover between the beta and alpha members, it would be anticipated that $A^b$-P/Df *a*-X hemizygous individuals, in which the homologue is deficient for a segment of the chromosome including the *A* locus and in which, therefore, the opportunity for synapsis of the beta:alpha complex and hence for exchanges between homologues in that region is removed, would yield no alpha offspring.

The data presented in Table 2 are pertinent to this argument; they concern both $A^b$-Lima and $A^b$-Cusco in hemizygotes involving *a*-X1 and *a*-X3, which are of X-ray origin (STADLER and ROMAN 1948) and are known to be deficient for a

TABLE 2

*Alpha derivatives from hemizygous* $A^b$-*P/Df* a-*X* individuals*

| Source | $A^b$-P gametes tested | Distribution of alpha strands among offspring |
|---|---|---|
| *T* $A^b$-Lima *Sh/N a*-X1 | 161,240 | 109 *T a Sh* |
| *T* $A^b$-Cusco *Sh/N a*-X1 | 400 | 1 *T a Sh* |
| *T* $A^b$-Lima *Sh/N a*-X3 | 45,480 | 29 *T a Sh* |
| *T* $A^b$-Cusco *sh/N a*-X3 | 2,350 | 3 *T a sh* |
| No proximal marker: | | |
| *N* $A^b$-Lima *Sh/N a*-X1 | 128,280 | 83 *N a Sh* |
| *N* $A^b$-Cusco *Sh/N a*-X1 | 86,355 | 44 *N a Sh* |
| *N* $A^b$-Lima *Sh/N a*-X3 | 4,555 | 4 *N a Sh* |
| *N* $A^b$-Cusco *Sh/N a*-X3 | 2,500 | 1 *N a Sh* |
| Totals | 431,160 | 274 |

* The deficient segments of both Df *a*-X1 and Df *a*-X3 include the *A* locus and the *Sh* locus, as explained in the text.

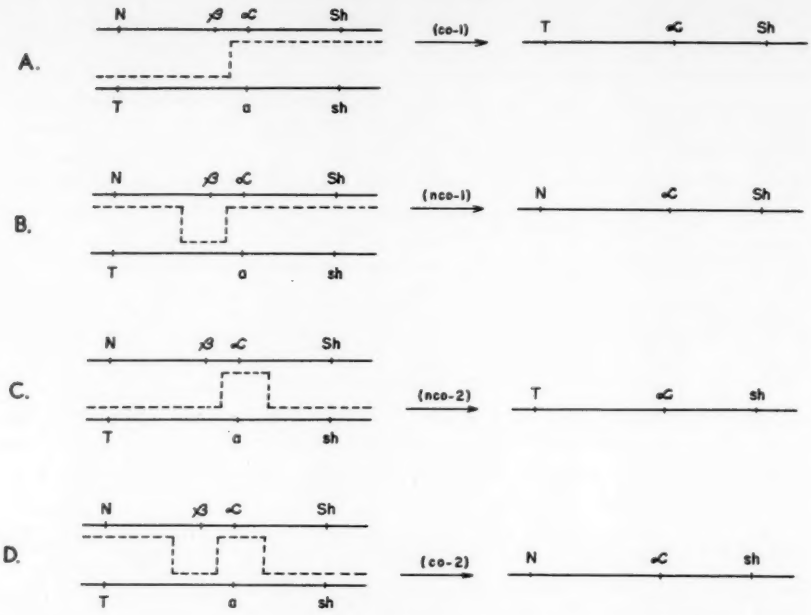



FIGURE 1.—Theoretical expectations for types of alpha-carrying strands from $A^b$-P/*a* heterozygotes on the multiple-exchange hypothesis. According to this scheme, all alpha isolations are attributable to the primary event, a crossover between beta and alpha members, held to occur when a relatively short segment involving the *A* region is effectively paired. The various combinations for marker loci among the alpha strands are functions of the number and position of additional exchanges (secondary events) within the effectively paired region. Complementary strands are not shown but, since the studies reported here deal with single strand analysis, it makes no difference whether the events diagrammed above result from switches on a copy-choice model or from exchanges between previously duplicated strands. The systematic basis for nco and co designation of alpha-carrying strands with regard to marker combinations is explained in a footnote to Table 3.

segment including the *A* locus and extending to the right beyond the *sh* locus. Contrary to expectation, these hemizygotes yield alpha progeny. Since these alpha derivatives arise from plants heterozygous for a deficiency in which there was no opportunity for exchange within the $A^b$ complex, it may be concluded that they could not have occurred as a result of multiple exchange. Data to be presented elsewhere indicate that nonrecombinant alpha cases are at least as frequent among the progeny of these deficiency heterozygotes as they are among the offspring of sib $A^b$-P/*a* heterozygotes. Thus, while the results presented in Table 2 can not be taken to indicate that multiple exchanges, as postulated, do not occur in maize, they clearly indicate that some other mechanism must be responsible for the occurrence of the vast majority of nonrecombinant alpha derivatives.

We shall come later to the independent question of whether the multiple exchange hypothesis is valid for maize.

Although both *a*-X1 and *a*-X3 occurred among the progeny of X-ray treated pollen carrying *A*, and the genetic evidence indicating that they are deficiencies is decisive, it has not been possible to identify them cytologically. Moreover, there is no known proximal marker sufficiently close to *A* to define accurately the left-most limits of these deletions. We have therefore made appropriate crosses to analyze for alpha occurrences among the progeny of a special type of hypoploid in which one of the chromosome 3 homologues is deficient for almost all of the long arm of chromosome 3 and the other is involved in the translocation 2-3d interchange. Owing to nondisjunction in the second division of the microspore, hypoploid individuals occur regularly among the progeny of pollen parents carrying reciprocal translocations involving the A and B type chromosomes (ROMAN 1947). The hypoploid of interest here (Figure 2) was produced by crossing homozygous *T* $A^b$-Lima *Sh* egg parents with pollen parents carrying the A-B translocation known as T-B3a, in which almost all of the long arm of chromosme 3 is translocated to the centric portion of the B chromosome. These plants are easily distinguished from their genetically balanced sibs since they are poor in vigor, are about one third to one half normal height, and exhibit characteristic morphological deviations from normal. Moreover, they show the high frequency of abortion (70–80 percent) of both pollen and ovules predicted from their unusual chromosome constitution. It should be emphasized that the *Tβ*:*αSh* segment of these hypoploids (see Figure 2) has no homologous segment with which to pair and that the $2^3$ chromosome, of which it is a part, is expected to be involved exclusively in synapsis with the chromosome 2 homologue.

Yet, among the relatively small population of 6,900 tested $A^b$-Lima gametes from backcrosses of these hypoploids with homozygous *N a sh* pollen, there were five alpha occurrences. Further analysis has confirmed that these cases are legitimate and that all are of the expected nonrecombinant type: *T α Sh*. Although the cases are too few to provide a reliable estimate of the frequency of the event, the rate as it stands compares favorably with that of alpha cases from the *a*-X hemizygotes (Table 2). The occurrence of alpha derivatives from this hypoploid, in which crossovers in the *T β*:*α Sh* segment are not possible, is critical evidence

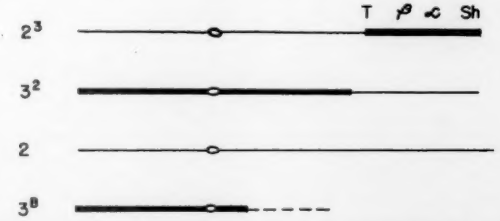



FIGURE 2.—Diagrammatic representation of the hypoploid individual in which one of the chromosome 3 homologues is deficient for most of the long arm of chromosome 3. See text for details.

indicating that the primary crossover event assumed on the multiple-exchange hypothesis is not required for nonrecombinant alpha isolation.

These results have a bearing also on the validity of the side-chain mechanism for the origin of nonrecombinant alpha cases. The more conservative version of this hypothesis, according to which the beta- and alpha-carrying segments are adjacent members in a single side chain, is illustrated in Figure 3. According to this scheme the primary event, leading to alpha isolation, is an exchange (or a switch on the copy-choice model) involving the side chains of homologous chromosomes, whereas the secondary events, tending toward the randomizing of marker combinations, are considered to be crossovers occurring coincidentally between the backbones of homologues. We see from Figure 3 that regardless of the sequence assumed for alpha and beta in the side chain, the corresponding side chain of the homologue must be involved in the alpha isolation. From the data presented above having to do with alpha occurrences from the $A^b$-P/$a$-X hemizygotes (Table 2) and from the T-B hypoploid, neither of which provides the required homologous side chain, it is obvious that the conservative side-chain hypothesis can not be the basis for the occurrence of the great majority of alpha cases.

The modified version of the side-chain hypothesis would have the alpha and

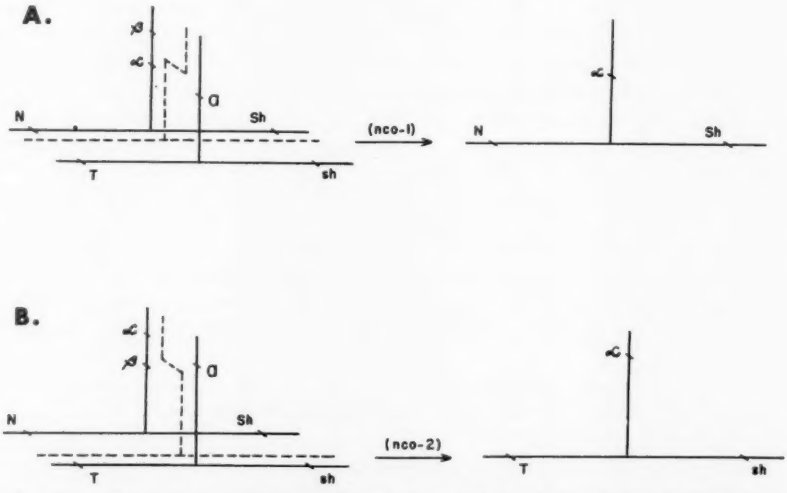



FIGURE 3.—Diagrammatic representation of the $A^b$-P/$a$ heterozygote on the conservative side-chain hypothesis, with alpha and beta members occupying the same side chain. Alternative models shown here differ in regard to sequence of alpha and beta in the side branch, but in both cases the primary event leading to alpha isolation requires the participation of the homologue carrying recessive *a*. These diagrams show only one of the two duplicating strands, and coincidental backbone exchanges (secondary events), leading to various combinations of markers, are also omitted. For simplicity, the *sh* marker locus, which on this hypothesis would also occupy a side chain, is placed in the backbone.

beta segments in separate but adjacent side branches. On this model, spatial separation of these elements permits the isolation of alpha by a primary event (crossover) in the backbone (Figure 4A) and, since alpha can not be isolated by the reciprocal backbone event, this scheme accommodates the observation, to be noted in the next section, that one of the two types of alpha recombinants is far more frequent than the other. Assuming that the side branch carrying the recessive *a* allele may pair with either the beta or alpha segment, this model also permits isolation of alpha by two additional types of primary event, each involving the side chains of both homologues (Figures 4B and 4C). However, since these three possibilities for alpha isolation involve participation of both homologues in the *A* region, it is obvious that they can not explain the occurrence of

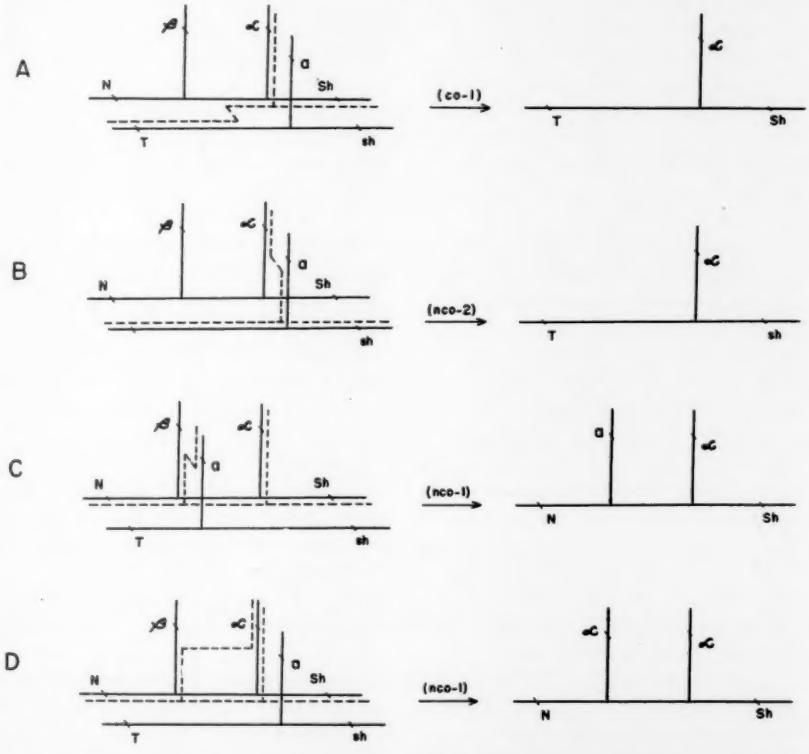



FIGURE 4.—Schematic representation of the $A^b$-P/*a* heterozygote on the modified side-chain hypothesis, with beta and alpha in adjacent side branches. Alpha isolation may occur by primary event in the backbone (A), or at the side-chain level (B, C, and D). Secondary events are not shown here, and only one of the duplicating strands is illustrated. As a matter of convenience, the *sh* marker is placed in the backbone rather than in a branch.

nonrecombinant alpha derivatives from the deficiency heterozygotes discussed above.

In fact, among the various mechanisms based on models presented here, only one (Figure 4D) will account for the occurrence of alpha derivatives without involving the homologue. It requires the assumption that the beta and alpha segments are homologous, and that copy-choice events (or exchanges) may take place between the adjacent beta and alpha side chains. Though this supposition may at first appear to be unwarranted, there is some justification for considering it a legitimate interpretation since there is independent evidence (LAUGHNAN 1955a,b) indicating that the beta and alpha members of both $A^b$-Ec and $A^b$-P are tandem, serial duplications. The event illustrated in Figure 4D would satisfy the evidence so far presented, since it is expected to yield nonrecombinant alpha isolations ($\alpha$:$\alpha$) by a mechanism not requiring participation of the homologue. However, this hypothesis may be considered valid only if the other types of alpha isolations illustrated in Figures 4A, 4B and 4C are realized among progeny of $A^b$-P/$a$ heterozygotes.

To summarize then, since the multiple-exchange hypothesis and the conservative side-chain hypothesis require the involvement of the homologue in the primary event they are not adequate to explain the nonrecombinant alpha cases among the progeny of $A^b$-P/$a$-X and T-B3a hypoploid individuals, but this does not necessarily eliminate them as explanations for alpha cases from nondeficient $A^b$-P heterozygotes or homozygotes. The modified side-chain hypothesis, which allocates alpha and beta to separate side chains, will account for nonrecombinant alpha occurrences from the deficiency heterozygotes but is acceptable only if certain other types of alpha isolations, predicted on this model, are obtained from nondeficient heterozygotes. In the analysis which follows, data on alpha isolates from various $A^b$-P heterozygotes are considered for their bearing on the validity of these several hypotheses.

### *Evidence from* A$^b$-*P heterozygotes*

The data reported in this section are from balanced heterozygotes in which the homologue of the $A^b$-P chromosome carries various other alleles. Unlike the hemizygotes dealt with in the foregoing section, these heterozygotes offer no impediment to occurrence of the primary event responsible for alpha isolation, and our concern here is with the distributions of marker combinations among alpha isolates and particularly with the fit of these distributions to predictions based on the proposed models.

Table 3 summarizes the data on alpha isolations from $A^b$-P/$a$ heterozygotes marked in various combinations with *T* and *sh* and with *T* and *et*. The 358 alpha isolates from *T* and *sh* marked sources (data from *T* and *et* marked parents will be discussed at a later point) fall into only two of the four possible marker classes. The most frequent type (nco-1) carries the nonrecombinant markers of the parental $A^b$-P complex. The other type, designated co-1, carries the shrunken marker of the parental $A^b$-P chromosome and the *T* marking of its homologue; this recombinant accounts for over 20 percent of the alpha strands from $A^b$-Lima

TABLE 3

*Constitutions of alpha-bearing strands from* $A^b$-*P/*a *individuals marked with* T *and* sh *or with* T *and* et

*T* 7.1 *β*:*α* 0.25 *Sh* 12.8 *Et*

| Source | $A^b$-P gametes tested | Number and distribution of alpha strands among offspring nco-1* | nco-2* | co-1* | co-2* |
|---|---|---|---|---|---|
| *N* $A^b$-Lima *Sh/T a sh* | 30,390 | 20 *N α Sh* | 0 *T α sh* | 4 *T α Sh* | 0 *N α sh* |
| *N* $A^b$-Lima *sh/T a Sh* | 53,190 | 24 | 0 | 12 | 0 |
| *T* $A^b$-Lima *Sh/N a sh* | 462,810 | 185 | 0 | 46 | 0 |
| Totals | 546,390 | 229 | 0 | 62 | 0 |
| *N* $A^b$-Cusco *Sh/T a sh* | 38,410 | 14 | 0 | 3 | 0 |
| *N* $A^b$-Cusco *sh/T a Sh* | 56,950 | 13 | 0 | 22 | 0 |
| *T* $A^b$-Cusco *Sh/N a sh* | 1,750 | 1 | 0 | 1 | 0 |
| *T* $A^b$-Cusco *sh/N a Sh* | 18,760 | 6 | 0 | 7 | 0 |
| Totals | 115,870 | 34 | 0 | 33 | 0 |
| *T* $A^b$-Lima *Et/N a et* | 296,130 | 109 | 1 | 43 | 9 |

* As illustrated by the strand constitutions provided in the first row of this table, here and in similar tables to follow, nco-1 refers to a strand carrying the nonrecombinant markers of the parental $A^b$-P chromosome; nco-2 refers to a strand carrying the parental markers of its homologue; co-1 refers to a recombinant strand that carries the distal marker of the parental $A^b$-P chromosome and the proximal marker of its homologue; and co-2 refers to a recombinant strand that carries the proximal marker of the parental $A^b$-P chromosome and the distal marker of its homologue.

and for almost half of those from $A^b$-Cusco heterozygotes. Since the marked segment is less than eight units it is apparent that the recombinant event is not independent of the event that isolates alpha. Thus the alpha isolates of co-1 type are consistent with the argument that beta and alpha are left and right members respectively of the parental complex and that alpha may be isolated by a crossover (primary event), but this of course begs an explanation of the far more frequent nonrecombinant nco-1 type.

According to the multiple-exchange hypothesis which assumes that the primary event is a crossover between beta and alpha members (Figure 1A) the nco-1 type strand is the result of a second crossover to the left of beta (Figure 1B), thus reconstituting the parental marker combination. We note, however, that this model also calls for the occurrence of nco-2 type alpha isolations (Figure 1C) as a result of the alternative type of double exchange having the second crossover between *α* and *sh;* and co-2 alpha strands are expected to result from triple exchanges (Figure 1D). Yet neither of these types is represented in the data. It is interesting to note that in the investigations on the *ad8* cistron in Aspergillus (PRITCHARD 1960a,b), where the marked segments *y*–0.2–*ad*–6.0–*bi* are almost identical with the *T*–7.1–$A^b$–0.25–*sh* segments in maize, all four types of marker combinations were found among the adenine prototrophs and were attributed to single and multiple exchanges within a localized pairing region whose mean length is estimated to be 0.4 of a conventional map unit. It is conceivable that in maize there is a lower probability of exchange within the hypothetical localized pairing segment (PRITCHARD calculates 0.6 for the *ad8* region in Aspergillus). On this argument the absence of nco-2 and co-2 alpha strands is attributable

to the rarity of secondary crossover events in the relatively short *α-sh* segment. The data in the last row of Table 3 concerning alpha strands from *T* and *et* marked $A^b$-Lima parents are pertinent to this question since the near equality of the marked segments in this heterozygote (this time the distal segment is longer than the proximal) should, on the multiple-exchange hypothesis, lead to the isolation of equivalent numbers of double crossover nco-1 and nco-2 alpha strands. However, this expectation is not realized; the nco-1 type strands far exceed the nco-2 type, of which there is only one, and moreover, even the co-2 class, which on this hypothesis is attributable to a triple crossover, is more frequent than the nco-2 type.

Data from other types of $A^b$-P heterozygotes indicate that the distribution of recombinants among alpha progeny noted in Table 3 is not specifically identified with the $A^b$-P/*a* heterozygote. Table 4 summarizes the relevant data on strand constitutions of 27 alpha isolates from $A^b$-Lima and $A^b$-Cusco heterozygotes in which the homologue carries the standard *A* allele; and similar analyses of 78 alpha strands from heterozygotes in which isolated beta members (LAUGHNAN 1961) are carried in the homologue, are given in Table 5. Among the total of 105 alpha strands obtained in these two experiments, 81 are of the nco-1 type but there was not a single case of the nco-2 type nonrecombinant which, according to the multiple-exchange hypothesis, is expected to be equally frequent, or nearly so.

TABLE 4

*Constitutions of alpha-bearing strands from* $A^b$-*P*/A *individuals marked with* T *and* sh *or with* T *and* et

| Source | $A^b$-P gametes tested | Number and distribution of alpha strands among offspring nco-1 | nco-2 | co-1 | co-2 |
|---|---|---|---|---|---|
| *N* $A^b$-Lima *sh/T A Sh* | 5,460 | 2 | 0 | 0 | 0 |
| *T* $A^b$-Lima *Sh/N A sh* | 19,225 | 4 | 0 | 1 | 0 |
| *N* $A^b$-Cusco *sh/T A Sh* | 22,600 | 9 | 0 | 2 | 0 |
| *N* $A^b$-Lima *Et/T A et* | 4,000 | 3 | 0 | 0 | 0 |
| *N* $A^b$-Lima *et/T A Et* | 550 | 2 | 0 | 0 | 0 |
| *T* $A^b$-Lima *Et/N A et* | 585 | 1 | 0 | 1 | 0 |
| *N* $A^b$-Cusco *Et/T A et* | 2,075 | 2 | 0 | 0 | 0 |
| Totals | 54,495 | 23 | 0 | 4 | 0 |

TABLE 5

*Constitutions of alpha-bearing strands from* T *and* sh *marked heterozygotes involving the* $A^b$-*Lima complex and isolated beta elements*

| Source | $A^b$-P gametes tested | Number and distribution of alpha strands among offspring nco-1 | nco-2 | co-1 | co-2 |
|---|---|---|---|---|---|
| *T* $A^b$-Lima *Sh/N* β-Lima *sh* | 98,520 | 38 | 0 | 11 | 0 |
| *T* $A^b$-*Lima Sh/N* β-Cusco *sh* | 49,135 | 20 | 0 | 9 | 0 |
| Totals | 147,655 | 58 | 0 | 20 | 0 |

It is concluded that multiple exchanges within localized regions of pairing which we have seen cannot account for the nonrecombinant alpha isolations from hemizygotes, are also an unsatisfactory explanation for the occurrence of nonrecombinant alpha derivatives from balanced heterozygotes. It is obvious that recombination in the *A* region of chromosome 3 in maize does not follow the pattern of negative intereference which PRITCHARD (1960a) proposed for Aspergillus and has generalized to other organisms (1960b).

The hypothesis of localized pairing segments was suggested to account for the occurrence of apparent multiple exchanges (negative interference) in short segments. Since the data presented here indicate either an absence or extreme rarity of such multiple exchanges, there is reason to doubt that crossing over in maize takes place in localized regions of intimate pairing; the hypothesis will accommodate the data only if it is assumed that withing such a pairing segment there is sufficient positive interference to prevent the occurrence of more than a single exchange, and under these circumstances there is no compelling reason to favor the hypothesis as an alternative to the conventional view.

The conservative side-chain hypothesis, which we have seen can not account for alpha isolations from deficiency heterozygotes, predicts (Figure 3) that among the alpha progeny of $A^b$-P heterozygotes one of the two possible nonrecombinant types for markers should predominate. The data of Tables 3, 4, and 5 show an overwhelming majority of the nco-1 class and would seem to indicate that on this hypothesis the alpha locus is proximal to beta in the side chain (Figure 3A). Since on this model the primary event that isolates alpha is exclusively a side-chain phenomenon it is expected that the marker constitutions of alpha isolates will be determined solely by the pattern of coincidental backbone crossovers within the paired segment straddling the *A* locus. If this pairing segment is short relative to the marked segments, as assumed by the hypothesis of localized pairing, the distribution of these coincidental exchanges on either side of the point of attachment of the alpha-beta side chain is expected to be random; if, alternatively, the length of pairing segment approaches or exceeds the length of the marked segments themselves, as on the conventional view, the distribution of coincidental crossovers to left and right of the alpha-beta side chain should be proportional to the lengths of the marked segments themselves.

Inspection of the data in Tables 3, 4, and 5 indicates that neither of these predictions is satisfied. Considering only the recombinant classes for marker loci we note that the ratios for co-1:co-2 recombinants among alpha strands from *T* and *sh* marked $A^b$-Lima and $A^b$-Cusco parents, respectively, are 83:0 and 35:0, and that the corresponding ratio for *T* and *et* marked $A^b$-Lima individuals is 44:9. These results are clearly in conflict with the expectation, based on a restricted pairing segment, of near equality of the two classes. The data from *T* and *sh* marked parents are not in disagreement with predictions based on the assumption that all of the marked region is available for exchange since in this instance only 3.4 percent of the recombinants are expected to fall in the co-2 class (the *a–sh* map value, 0.25, represents 3.4 percent of the *T–sh* segment, 7.35). However, a similar calculation for the *T–et* segment leads to the expectation that 65 percent

of the recombinant alpha strands should be of the co-2 type (the *a–et* map value, 13.1, is about 65 percent of the *T–et* segment, 20.2). But the data in the last row of Table 3 are in disagreement with this prediction since only nine (17 percent) of the 52 recombinant strands are of the co-2 type. Thus, regardless of the length we assume for the effective pairing segment, the actual data on frequencies of recombination among alpha isolates are contradictory and lead to the conclusion that the conservative side-chain hypothesis is unacceptable.

As noted in a previous section, the modified side-chain hypothesis, which assumes that alpha and beta are carried on separate side chains, is the only scheme among the ones considered here that will satisfactorily account for nonrecombinant alpha occurrences from deficiency heterozygotes. Moreover, if the sequence of alpha and beta side chains is $T\ \beta{:}\alpha\ sh$, as represented in Figure 4, the occurrence of nco-1 and co-1 alpha recombinants from nondeficient $A^b$-P heterozygotes (Tables 3, 4, and 5) is anticipated; the former (nco-1) are interpretable as primarily the result of an event involving the beta and alpha side chains of the $A^b$ homologue (Figure 4D), whereas the latter (co-1) are expected to result primarily from backbone crossovers of one type (Figure 4A) between the beta and alpha side chains. However, according to this scheme, $A^b$-P heterozygotes are expected to yield alpha derivatives as a result of two additional primary events, both occurring at the side-chain level. One of these involves a switch in copy (or exchange) between homologous side chains carrying the beta and the recessive *a* elements (Figure 4C) and is expected to yield occasional nco-1 strands carrying the $a{:}\alpha$ type complex; the other involves the alpha side chain in a corresponding event and leads to isolations of alpha in nco-2 type strands (Figure 4B). The data are clearly not in agreement with the latter prediction since among the 625 alpha isolations reported in Tables 3, 4, and 5, only one nco-2 type strand was isolated and this occurred among the progeny of a *T* and *et* marked parent. The $a{:}\alpha$ type complex, whose isolation in nco-1 type strands is anticipated from the alternative event, should exhibit the dotted type of mutability since it carries the recessive *a* allele which mutates to *A* in the presence of *Dt* (RHOADES 1938). Mutable alpha derivatives have been identified but have occurred almost exclusively among the co-1 class, indicating that the occurrence of the $a{:}\alpha$ complex is attributable to conventional crossing over rather than to side-chain events.

Purely hypothetical considerations (WINGE 1955; SCHWARTZ 1955; TAYLOR 1957) suggest an endless array of possible variations on the branched chromosome model. If, however, the two alternative schemes treated here may be considered to incorporate the basic recombinational features of side-chain models in general, the data justify the conclusion that the anomalous, nonrecombinant alpha isolations are not attributable to primary events in side chains. Indeed, the evidence from these studies is opposed to the view that genetic material in maize is located in branches of the chromosome that offer the opportunity for recombination at that level.

### *Evidence from inversion-3a heterozygotes*

These studies deal exclusively with alpha derivatives from inversion hetero-

zygotes in which one chromosome 3 member, either normal or interchanged, carries the beta:alpha complex and the other carries the recessive *a* allele in the differential segment of the paracentric inversion-3a. The breakpoints of this inversion have been placed on the cytological map of the long arm of chromosome 3 at 3L 0.4 and 3L 0.95 (RHOADES and DEMPSEY 1953; personal communication); the proximal point of exchange is to the left of $lg_2$ (liguleless-2) and the distal break is to the right of *et*, such that the *lg*, *T*, *a*, *sh* and *et* loci are all within the inverted segment which includes a minimum of 61 units of the genetic map. The distal breakpoint of this inversion is about 15 map units beyond *A*, which means that this segment represents about one fourth of the genetic length of the inversion. As expected, balanced products of single crossovers within the inverted segment are not found among the backcross progeny of plants heterozygous for this inversion, but double crossover strands are recovered in 1.1 percent of functional male gametophytes and in 1.8 percent of functional female gametophytes (RHOADES and DEMPSEY 1953).

Inversion-3a heterozygotes offer an ideal opportunity to test the hypothesis of multiple exchange since strands involved in a single exchange or in odd numbers of exchanges within the inverted segment of these heterozygotes will not be represented among the offspring. Accordingly, the products of single exchanges between the beta and alpha members, which ordinarily would be scored as recombinant alpha strands, should be eliminated from the progeny of these individuals. But according to the hypothesis of localized pairing, which would account for the alpha nonrecombinant class as the result of double exchanges in a short, effectively paired segment spanning the *A* locus, the $A^b$-P inversion heterozygotes should yield nonrecombinant alpha progeny. The expected distribution of these nonrecombinant alpha strands may be deduced by a consideration of the events illustrated in Figure 5 which gives a diagrammatic representation of the inversion heterozygote in which the beta:alpha complex is carried in a noninterchanged

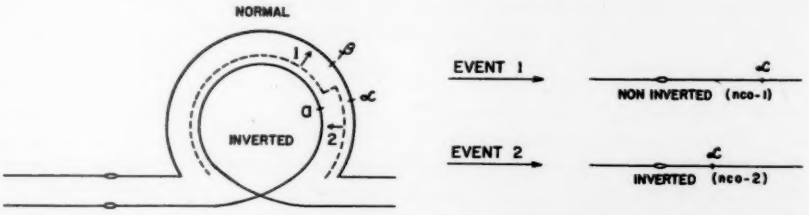



FIGURE 5.—Pairing configuration in the heterozygous, paracentric inversion-3a individual with the $\beta$:$\alpha$ complex in the normal chromosome and recessive *a* in the inverted homologue. A primary event which isolates alpha is illustrated by the dashed strand, and additional exchanges involving this strand and occurring either to the left (event 1) or to the right (event 2) are illustrated with arrows. If, as on the multiple-exchange hypothesis, the effective pairing region is short, events 1 and 2 are expected with equal frequencies and should lead to the isolation of equivalent numbers of nco-1 and nco-2 alpha isolations shown at the right. For the sake of simplicity the exchanges are diagrammed as copy choice events but the predictions are the same if exchanges between previously duplicated strands are assumed.

chromosome 3. Assuming the initial occurrence (primary event) of a switch in copy (or an exchange) between beta and alpha, a second such event within the differential segment may be either to the left (event 1) or right (event 2) of the $A^b$ complex. As shown in Figure 5, the first of these is expected to isolate alpha in a noninverted strand (nco-1) whereas the second should lead to alpha isolations in the inverted homologue (nco-2). If, according to the multiple-exchange hypothesis, the effective pairing region is short relative to the segments located to the left and right of the complex and within the inversion, these two types of nonrecombinant alpha strands are expected to be equally frequent. However, if the frequencies of the two types of secondary crossovers are held to be proportional to the lengths of the marked segments within which they may occur, the nco-1 type alpha strand is expected to be three or four times more frequent than the nco-2 type; and if account is taken of the likely effect of chromosomal interference the discrepancy between the frequencies of these two types should be even greater.

The distributions of alpha derivatives among backcross progenies of $A^b$-P inversion heterozygotes are given in Table 6. It should be noted that the allocation of an alpha strand to the nco-1 or nco-2 class in this instance does not require gene markers since there is no difficulty in making this determination on the basis of pollen abortion in the two types; thus, individuals which are heterozygous for an alpha-carrying strand of nco-2 type (inverted) and a normal *a* strand contributed by the tester parent, should regularly exhibit from 15 to 20 percent pollen abortion, while the nco-1 type plant, which carries two normal chromosomes 3, is expected to have normal pollen. The data in the first two rows of Table 6, which deal with alpha strands derived from inversion heterozygotes in which the $A^b$-Lima complex is carried in a noninterchanged chromosome, are not in agreement with the multiple exchange hypothesis since there were 67 isolations of the nco-1 type and not a single case of the nco-2 type alpha strand predicted on this model. However, even though there were no nco-2 type alpha derivatives among the progeny of these inversion heterozygotes, it is not unlikely that some of the nco-1 alpha isolates represent double crossovers of the conventional type

TABLE 6

*Alpha derivatives from heterozygotes carrying* $A^b$*-P in a normal or interchanged chromosome and recessive* a *in the inverted segment of Inversion-3a*

| Source | $A^b$-P gametes tested | Distribution of alpha strands among offspring: Noninverted (nco-1) | Inverted (nco-2) |
|---|---|---|---|
| *N* $A^b$-Lima/In 3a: *a* | 51,930 | 56 *N a* | 0 |
| *N* $A^b$-Lima *et*/In 3a: *a Et* | 8,400 | 11 *N a et* | 0 |
| *T* $A^b$-Lima/In 3a: *a** | 26,880 | 12 *T a* | 0 |
| *T* $A^b$-Lima *et*/In 3a: *a Et* | 17,060 | 7 *T a et* | 0 |
| *T* $A^b$-Lima *sh*/In 3a: *a Sh* | 4,220 | 2 *T a sh* | 0 |
| *T* $A^b$-Cusco *sh*/In 3a: *a Sh* | 37,870 | 23 *T a sh* | 0 |

* In this and the three following heterozygotes, the $A^b$-P complex is carried in a $2^s$ interchanged, noninverted chromosome, and recessive *a* is carried in the inverted, noninterchanged chromosome 3.

since there is a relatively long region to the left of the primary event, but still within the differential segment of the inversion, available for the secondary event.

The frequency of nonrecombinant alpha occurrences (*ca.* one per 900 tested gametes) from these inversion heterozygotes is higher than that for any genotype we have tested so far. Since it may be calculated from data in the original report (RHOADES and DEMPSEY 1953) that double crossovers within the differential segment of inversion-3a heterozygotes are realized with a frequency only about one fifth of that expected for corresponding events in noninversion heterozygotes, the observed high frequency of alpha occurrences in these studies is itself at odds with the argument that the nonrecombinant alpha occurrences are primarily attributable to double crossovers of the conventional type.

The data in the last four rows of Table 6 may be taken as further evidence against the multiple-exchange hypothesis. The alpha strands recovered here derive from inversion heterozygotes in which the $A^b$ complex is carried in an interchanged $2^s$, noninverted chromosome. If it is assumed that effective pairing segments are highly localized, consideration of the rather complicated configuration expected in these heterozygotes leads to the expectation of equal numbers of nco-1 (50 percent aborted pollen in this instance) and nco-2 (15 to 20 percent aborted pollen) alpha derivatives. And if the effective pairing segment is relatively extended, as on the conventional view, it can be shown that these heterozygotes should still yield nearly equivalent numbers of alpha strands of these two types, in addition to a third class carrying alpha on a reconstituted normal chromosome 3. However, the pertinent data in Table 6 indicate that alpha isolations from these heterozygotes occur exclusively in association with nco-1 type strands.

These observations support the conclusion, reached on the basis of evidence from hemizygotes and balanced heterozygotes, that the hypothesis of multiple exchange in localized regions of pairing is not a satisfactory explanation for the occurrence of the nonrecombinant alpha derivatives. Beyond this, the evidence from inversion heterozygotes suggests that the mechanism involved in the isolation of nonrecombinant alpha derivatives does not require exchange between homologues, a conclusion which was first suggested by the evidence from deficiency heterozygotes.

The data on alpha isolations from inversion heterozygotes should be useful in testing predictions based on the side-chain hypotheses. We presume that the backbone of the chromosome, but not the side chain, is involved in the breaks and rejoins required to produce an inversion and that the complications which arise following certain backbone events in individuals heterozygous for the inversion should not attend the hypothetical occurrences that we pose at the level of the side branches. Thus, while the vast majority of backbone crossovers occurring in the differential segment of inversion heterozygotes should not be realized among their progeny, the products of hypothetical side-chain events from these same individuals should not be reduced in frequency among the offspring, except to the extent that (a) mechanical hindrance to pairing in the inversion

heterozygote may reduce the initial frequency of side-chain events between homologues, and (b) the side-chain event is correlated positively with backbone events which would lead to disproportionate elimination of the former from the progeny.

Referring once more to Figure 3A we note that on the conservative side-chain hypothesis, the primary event isolates alpha on the side chain of a strand carrying the parental markers of the $A^b$-P chromosome (nco-1). Since, on this model, alpha and beta reside in the same branch only one type of primary event is possible. In inversion heterozygotes of the type illustrated in Figure 5 this event should lead to isolations predominantly of the nco-1 type in which the alpha element is carried in a normal chromosome. Alpha strands with single coincidental crossovers in the inversion would be eliminated and the only basis for the isolation of alpha in the inverted homologue (nco-2) would be a coincidental double crossover in adjacent regions of the backbone. While, except for the absence of the nco-2 class, the data given in Table 6 are not seriously in conflict with this scheme, it should be recognized that the test involved here is not an exacting one and that the more critical evidence from hemizygotes and balanced heterozygotes could not be accommodated on this hypothesis. Moreover, it is significant to note (see Figure 3A) that if the *a* member is assumed to pair indiscriminately with alpha or beta in the homologous side branch, the side-chain event in the latter instance should isolate an $\alpha{:}a$ complex which, as we have already seen, is expected to mutate in response to the *Dt* gene. Forty-seven of the 111 alpha isolations from inversion heterozygotes listed in Table 6 have been tested for *Dt*-induced mutability and all but one of these proved to be stable. The single mutable case may be considered the result of a primary crossover event isolating the $a{:}\alpha$ complex, accompanied by a coincidental exchange to reconstitute the nco-1 strand type.

The data from inversion heterozygotes afford a critical test of the modified side-chain hypothesis since according to this scheme side-chain events (Figures 4B, 4C, and 4D) in such heterozygotes (see Figure 5) should lead not only to $\alpha{:}\alpha$ and $a{:}\alpha$ isolates in the noninverted (nco-1) chromosome but also to nco-2 type derivatives in which alpha is carried in the inverted homologue. However, these predictions are not realized since, as already noted, nco-2 type alpha isolates are not represented among the progeny of inversion heterozygotes (Table 6) that produced 111 nco-1 type alpha derivatives. Moreover, as noted above, mutability tests of 47 of these derivatives indicate that only one is likely to have the constitution $a{:}\alpha$; as pointed out in the preceding paragraph, this case may be explained on conventional grounds as the result of a double crossover.

To summarize, the evidence from inversion heterozygotes, like that from hemizygotes and balanced heterozygotes, indicates that neither the multiple-exchange hypothesis nor the side-chain hypothesis is adequate to account for the origin of nonrecombinant alpha derivatives from the $A^b$-P complexes.

## DISCUSSION

The recent impetus lent to both the multiple-exchange and side-chain hypotheses clearly derives from the need to explain the seemingly contradictory, but

not infrequently encountered, evidence from several microorganisms that prototrophs, expected to arise as a result of crossover events, are too frequently isolated as nonrecombinants for marker loci. The experiments described here were undertaken to determine whether the nonrecombinant alpha derivatives from $A^b$-P complexes, which show similar deviations from the expected pattern, might reasonably be attributed to either of these mechanisms. The evidence leads to the conclusion that neither of these mechanisms will satisfactorily account for the exceptional alpha isolations. The writer is strongly inclined toward the further conclusion, which is not identical with the first, that the phenomena in question do not occur in maize, but it is apparent that certain considerations, aside from the one that these studies involve only one segment of the maize genome, argue in favor of a less sweeping statement.

The data from $A^b$-P/*a*-X hemizygotes and from the T-B hypoploid indicate that isolation of the alpha component is possible without participation of the homologue. The only reasonable conclusion to be reached from this finding is that some kind of intrachromosomal mechanism is involved in these alpha occurrences. However, unless the assumption is made that nonrecombinant alpha strands arise by only one mechanism, the evidence from the deficiency heterozygotes has no critical bearing on the validity of the multiple-exchange and side-chain hypotheses, since, assuming a multiple origin, this background would screen effectively against cases originating by either of these latter mechanisms, but would not be expected to eliminate, or even diminish, those cases arising by the intrachromosomal event. In this connection it should be noted that the frequency of nonrecombinant alpha occurrences is at least as high among the progeny of hemizygotes as among the offspring of sib, balanced heterozygotes (LAUGHNAN 1961). This observation favors a single origin (intrachromosomal) for these derivatives but, so far as the deficiency experiments are concerned, may be regarded as circumstantial evidence only since it could be argued that some kind of compensating influence is involved in the multiple origin of alpha exceptions.

Unlike the deficiency heterozygotes, the balanced $A^b$-P heterozygotes and the $A^b$-P inversion heterozygotes should pose little or no hindrance to the primary alpha-isolating events assumed for either the multiple-exchange hypothesis or the side-chain models. Nevertheless, the distribution of recombinant and nonrecombinant alpha strands from these backgrounds is inconsistent with the patterns predicted by these schemes, and the conclusion is warranted that they are not involved in the origin of the anomalous alpha derivatives.

It is perhaps more significant to note that if the phenomena of multiple exchange (high negative interference) and/or of recombination between hypothetical side branches of the chromosome occur in maize, certain critical types of alpha strands should be obtained among the progeny of the balanced and inversion heterozygotes. Since these were not realized we may conclude that (a) multiple exchanges within short chromosomal segments either do not occur in maize or occur so rarely that they were not apparent in the studies reported here, and (b) the assumption that the genetic material in maize, including the alpha

and beta elements, is oriented in side chains that represent lateral extensions of the chromosome backbone and are subject to recombinational events *inter se*, is untenable.

It is conceivable that the phenomenon of localized pairing may exist in maize even though there is no compelling reason, from the studies reported here, to assume it. PRITCHARD's analyses of recombination within the *ad8* cistron and nearby segments in Aspergillus, and his conclusions from these studies, may suggest that the hypothesis of localized pairing should not be abandoned at this time for maize even though it appears that in this material multiple exchanges are not realized within the hypothetical effectively paired region.

Occasional reference has been made in this presentation to an intrachromosomal mechanism involved in the origin of alpha nonrecombinants. The evidence on alpha derivatives from the deficiency heterozygotes clearly indicates that such a phenomenon is involved, and the results obtained from the balanced and inversion heterozygotes are consistent with this conclusion. Preliminary consideration has been given to such a mechanism in other publications (LAUGHNAN 1955c, 1957, 1961) and a detailed treatment is in preparation at this time.

## SUMMARY

A detailed analysis of the distributions of alpha-carrying strands among the progeny of $A^b$-P parents carrying the beta:alpha complex was undertaken. In particular, the fit of these distributions to expectations on the hypothesis of multiple exchange in localized regions of pairing, and on two alternative side-chain models, was investigated.

The yield of alpha derivatives from $A^b$-P/$a$-X deficiency heterozygotes and from T-B hypoploid individuals indicates that isolation of alpha from the beta:alpha complex does not require participation of the homologue, and indicates that the origin of the vast majority of alpha nonrecombinants is attributable to an intrachromosomal mechanism.

The distributions of recombinant and nonrecombinant alpha strands from various balanced $A^b$-P heterozygotes, and from individuals carrying the $A^b$-P complex in a normal or interchanged chromosome and the recessive $a$ allele in an inversion-3a homologue, are inconsistent with expectations on both the multiple-exchange hypothesis and the side-chain models.

It is concluded that (a) multiple exchanges within short chromosomal segments either do not occur in maize or occur so rarely that they were not apparent in the studies reported here, and (b) the assumption that alpha and beta, and presumably the genetic material in general in maize, are oriented in side chains that are subject to recombinational events is untenable.

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# THE LINKAGE MAP OF THE HOUSE FLY, MUSCA DOMESTICA L.[1]

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A TOTAL of nine house fly mutants which occurred spontaneously or were induced by X-ray were listed by two Russian investigators, SARKISSIAN (1941) and SEREBROVSKY (1941). These may have been the first attempts at the formal study of the genetics of *Musca domestica* L. Apparently these investigators did not continue and extend this work subsequently.

Recently, in connection with problems of resistance to insecticides, the importance of basic genetic studies of the house fly has gradually been recognized by many investigators. Morphological mutants are necessary as markers in studies designed to reveal the mode of inheritance of resistance to insecticides. To date a considerable number of house fly mutants have been found and assembled by the Institute of Zoology, University of Pavia, Italy (MILANI 1954, and other publications), by the Department of Genetics, Osaka University, Japan (TSUKAMOTO, OGAKI and KOBAYASHI 1957, and other publications) and by this laboratory (SULLIVAN and HIROYOSHI 1960; HIROYOSHI 1960). HIROYOSHI (1960) demonstrated the existence of all five autosomal linkage groups and numbered them. However, no mutant marker has yet been found for the sex chromosome. Independently TSUKAMOTO, BABA and HIRAGA (1961) classified their mutants into five linkage groups and, after receiving some mutant stocks from us, proved that their linkage groups correspond to the ones reported by the author.

Since more than two marker genes are now known for each chromosome (except the sex chromosome) the author has attempted to prepare a standard linkage map of the house fly, which should be of use to all workers on genetical problems in this organism.

## MATERIALS AND METHODS

Adult house flies of stock and experimental cultures were fed with milk diluted with water. Larvae were reared in a medium made from CSMA standard house fly medium, Karo syrup, baker's yeast and water. Both mass culture and single pair mating methods were used for linkage tests. A clutch of eggs from a single

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pair of flies was raised in a one-half-pint bottle which sufficed for raising up to 200 healthy flies, and eggs from a mass culture were reared in a one-quart jar in which up to 1000 larvae grew satisfactorily. All experiments were undertaken in a room maintained at a temperature of 80°F. Details on our rearing methods can be found in HIROYOSHI (1960) and SULLIVAN (in press).

The mutant markers studied in this paper are listed below. As details of all these mutants have already been described (HIROYOSHI 1960) only the symbol of the mutant, full name, linkage group and main phenotypic characteristic are listed here.

*bwb* : brown-body, II. Yellowish brown body color.
*car* : carnation, V. Bright red eye color.
*cm* : carmine, V. Bright red eye color.
*cp* : clump, II. Thickening of the $L_4$ wing vein at the posterior part.
*ct* : cut, IV. Wing tip cut.
*ge* : green, II. Greenish-yellow eye color.
*it* : interrupted, VI. Interruption of the $L_4$ wing vein.
*Lp* : Loop, III. Extra crossveins between the $L_3$ and $L_4$ wing veins.
*oc* : ocelliless, V. Missing ocelli and ocellar bristles.
*ocra* : ocra, III. Greenish-yellow eye color.
$ps^{s8k}$ : pigmented-sternites$^{s8k}$, V. Sternites of abdomen blackened.
*rb* : ruby, IV. Bright red eye color.
*ro* : rough eye, II. Eye surface roughened.
*sb* : subcostal-break, VI. Interruption of the subcostal vein of the wing.
*w* : white, II. White eye color.
$w^{s7}$ : white$^{s7}$, II. White eye color; an allele of *w*.
$w^{au}$ : white-auburn, II. Brown eye color; an allele of *w*.

Several wild type laboratory strains were used as normal type alleles of these marker genes, but they are merely referred to as normal type or by the + symbol without distinguishing their origins.

## CROSSES AND RESULTS

Prior to the linkage experiments, a great effort was made to synthesize mutant strains with more than two marker genes on a chromosome, so that the linkage tests could be done by the so-called "direct method". Then, in order to determine the crossing over ratios in male and female flies, the $F_1$ hybrids between normal type flies and the mutants carrying more than two marker genes on a chromosome were backcrossed to the parental mutant females and males, respectively. In most cases, crosses between a mutant male and a normal type female and also its reciprocal combination were set up to obtain $F_1$ heterozygotes for the tests. This was done to distinguish any cytoplasmic effects of a mutant and a normal type strain on crossing over ratios.

*Test for crossing over in the male fly:* Since the exceptional occurrence of crossing over in the male house fly had been reported by several investigators, extensive testcrosses to detect male crossing over were carried out. From among various experiments performed for this purpose, the results from three inde-

pendent tests with markers in the 2nd, 3rd and 5th linkage groups are presented (Table 1).

A triple recessive mutant combination, *bwb ge ro* (brown-body, green and rough eye), of the 2nd linkage group was tested first. Male hybrids from the cross between the mutant female and a normal type male and from the reciprocal cross were backcrossed to homozygous triple mutant females and the phenotypes of the progenies were examined. Among 7311 offspring all flies were either normal, + + +, or *bwb ge ro* phenotypes; no recombinants at all were found. Thus there is no evidence of crossing over in males in this experiment. The segregation ratio of + + + to *bwb ge ro* flies, however, does not fit the expected 1:1 ratio. The number of + + + male flies exceeds by far that of the *bwb ge ro* males, and the number of wild type females is much less than that of the mutant type females. This is due to the fact that some pairs of flies produce only *bwb ge ro* females and + + + males without any + + + female or the mutant type male. This kind of segregational abnormality, or sex-limited inheritance, has been observed very frequently when phenotypically normal males were crossed to mutant females of the 2nd linkage group. The peculiar phenomenon will be discussed in a later section.

A double mutant combination *ocra Lp* (ocra eye and Loop wing vein) of the 3rd linkage group was used to study crossing over in males between these two loci. The mutant *ocra* is a recessive, while *Lp* is dominant and lethal when homozygous. A *Lp* $F_1$ male from the cross between a normal type fly and the double mutant (genotype: *ocra Lp*/*ocra* +) was crossed to homozygous *ocra* females and its progenies were examined. The genotype of the tested hybrid male was *ocra Lp*/+ +; therefore, if crossing over occurred between these two

TABLE 1

*Linkage tests attempting to detect crossing over in the male house fly*

| Type of cross* | Phenotype of offspring | | Male | Female | |
|---|---|---|---|---|---|
| *bwb ge ro* ♀ × *bwb ge ro*/+ + + ♂ and *bwb ge ro* ♀ × + + +/*bwb ge ro* ♂ | Nonrecombinant | + + + | 2939 | 1198 | |
| | | *bwb ge ro* | 918 | 2256 | |
| | Recombinant | Others | 0 | 0 | |
| | | Total | (3857) | (3454) | (7311) |
| *ocra* ♀ × *ocra Lp*/+ + ♂ and *ocra* ♀ × + +/*ocra Lp* ♂ | Nonrecombinant | + + | 4078 | 3552 | |
| | | *ocra Lp* | 3918 | 3377 | |
| | Recombinant-like | *ocra* + | 8† | 2† | |
| | | Total | (8004) | (6931) | (14935) |
| *cm oc* $ps^{58k}$ ♀ × *cm oc* $ps^{58k}$/+ + + ♂ and *cm oc* $ps^{58k}$ ♀ × + + +/*cm oc* $ps^{58k}$ ♂ | Nonrecombinant | + + + | 1603 | 1399 | |
| | | *cm oc* $ps^{58k}$ | 1288 | 1201 | |
| | Recombinant | Others | 0 | 0 | |
| | | Total | (2891) | (2600) | (5491) |

* The numerator of a hybrid formula indicates the chromosome from maternal origin, and the denominator is of paternal origin.

† By further experiments it was revealed that these *ocra* phenotype flies were carrying *Lp* genes without expressing them.

loci, it would be detected as flies with phenotypes of *ocra* or *Lp*, but not both. Among 14,935 offspring examined, all were nonrecombinant types, either *ocra Lp* or wild phenotypes excepting ten apparently recombinant *ocra* flies. Further tests showed that these ten *ocra* flies were not the results of crossing over in the males. When these *ocra* phenotype flies were mated with wild type laboratory flies, *Lp* offspring were recovered in all ten cases. Thus the failure of *Lp* to appear in the testcross was not the result of crossing over in males, but merely indicates that the *Lp* gene has poor penetrance.

A triple recessive mutant *cm oc* $ps^{58k}$ (carmine eye, ocelliless and pigmented-sternites$^{58k}$) was used in another experimental attempt to detect male crossing over in the 5th chromosome. As shown in Table 1, no recombinants were found among 5,491 offspring from the backcross of males heterozygous for the three recessive markers to the homozygous mutant females.

Therefore, in all three experiments crossing over did not occur in male house flies. Besides the experiments described above, various kinds of crosses were performed with different markers or different combinations of markers, but without getting any indication of crossing over in the male.

*Linkage tests for female crossing over:* In order to obtain data for the linkage map, crossing over in female house flies was studied by crossing female hybrids from normal type flies and mutants carrying more than two marker genes on a chromosome to homozygous mutant males (Table 2). Each set of data shown in the table is the pooled result from more than five independent, single pair cultures and several mass cultures. Among these single pair cultures or mass cultures, or between single and mass cultures, there was no remarkable difference in results. In some cases, several different strains were used for linkage tests to determine a recombination value between two loci. With a few exceptions, the recombination values from these experiments using different strains coincide satisfactorily.

In the 2nd linkage group, the following five loci were studied: *w* (white eye), *bwb* (brown-body), *ge* (green eye), *ro* (rough eye) and *cp* (clump in the $L_4$ wing vein). They are all recessive markers. Two triple recessive mutant combinations, *w bwb cp* and *bwb ge ro*, made it possible to perform three-point linkage tests. From the results of these tests, gene arrangements in the orders of *w–bwb–cp* and *bwb–ge–ro* were revealed. A linkage test with a double recessive mutant, *w ro*, showed that these two loci were located far away from each other, and from these results together with those obtained from the three point tests the gene arrangement of the 2nd linkage group was determined as *w–bwb–ge–ro–cp*. The two recombination values between *w* and *bwb*, one from the experiment performed with the *w bwb* strain and the other with the *w bwb cp* strain, do not coincide very well. The reason of this disagreement is unknown. The over-all recombination value between *w* and *bwb* genes is 18.7. The recombination ratios between the *w* and *ro* loci and also between *w* and *cp* are more than 45 percent. If the occurrence of double crossing over and other disturbing factors are taken into consideration, these values are approximately what would be expected if the loci *w–ro* and *w–cp* are more than 50 map units apart. The distances for *bwb–ge*, *ge–ro* and *bwb–ro* were calculated from the results of the three point test of *bwb*

TABLE 2

*Linkage tests to determine genetic distances among some loci of the house fly*

| Linkage group | Loci tested | Type of cross* | Total number of offspring examined | Recombination value (percent) Single test | Pooled |
|---|---|---|---|---|---|
| II | *w–bwb* | *w bwb cp/+ + +* ♀ × *w bwb cp* ♂ | 4857 | 15.3 | |
| | | *+ bwb/w +* ♀ × *w bwb* ♂ | 10346 | 20.3 | (18.7) |
| II | *w–ro* | *w ro/+ +* ♀ × *w ro* ♂ | 4277 | ... | (45.2) |
| II | *w–cp* | *w bwb cp/+ + +* ♀ × *w bwb cp* ♂ | 4856 | ... | (46.4) |
| II | *bwb–ge* | *bwb ge ro/+ + +* ♀ × *bwb ge ro* ♂ | 13261 | 23.5 | |
| | | *+ + +/bwb ge ro* ♀ × *bwb ge ro* ♂ | 12806 | 25.3 | (24.4) |
| II | *bwb–cp* | *w bwb cp/+ + +* ♀ × *w bwb cp* ♂ | 4857 | ... | (34.2) |
| II | *bwb–ro* | *bwb ge ro/+ + +* ♀ × *bwb ge ro* ♂ | 13261 | 33.7 | |
| | | *+ + +/bwb ge ro* ♀ × *bwb ge ro* ♂ | 12806 | 33.4 | (33.5) |
| II | *ge–ro* | *bwb ge ro/+ + +* ♀ × *bwb ge ro* ♂ | 13216 | 11.9 | |
| | | *+ + +/bwb ge ro* ♀ × *bwb ge ro* ♂ | 12806 | 9.2 | (10.6) |
| III | *ocra–Lp* | *ocra Lp/+ +* ♀ × *ocra +* ♂ | 1108 | ... | (39.9) |
| V | *cm–car* | *cm car/+ +* ♀ × *cm car* ♂ | 4129 | 4.0 | |
| | | *+ +/cm car* ♀ × *cm car* ♂ | 12026 | 4.4 | (4.3) |
| V | *cm–oc* | *cm oc/+ +* ♀ × *cm oc* ♂ | 1034 | 44.1 | |
| | | *+ +/cm oc* ♀ × *cm oc* ♂ | 3005 | 44.2 | |
| | | *cm oc* $ps^{58k}$*/+ + +* ♀ × *cm oc* $ps^{58k}$ ♂ | 2842 | 43.1 | |
| | | *+ + +/cm oc* $ps^{58k}$ ♀ × *cm oc* $ps^{58k}$ ♂ | 5981 | 43.7 | (43.7) |
| V | *cm–*$ps^{58k}$ | *cm* $ps^{58k}$*/+ +* ♀ × *cm* $ps^{58k}$ ♂ | 1157 | 49.0 | |
| | | *+ +/cm* $ps^{58k}$ ♀ × *cm* $ps^{58k}$ ♂ | 3035 | 46.6 | |
| | | *cm oc* $ps^{58k}$*/+ + +* ♀ × *cm oc* $ps^{58k}$ ♂ | 2842 | 48.6 | |
| | | *+ + +/cm oc* $ps^{58k}$ ♀ × *cm oc* $ps^{58k}$ ♂ | 5981 | 49.1 | (48.6) |
| V | *car–oc* | *car oc/+ +* ♀ × *car oc* ♂ | 2691 | 40.6 | |
| | | *+ +/car oc* ♀ × *car oc* ♂ | 3588 | 40.0 | (40.3) |
| V | *oc–*$ps^{58k}$ | *cm oc* $ps^{58k}$*/+ + +* ♀ × *cm oc* $ps^{58k}$ ♂ | 2842 | 18.8 | |
| | | *+ + +/cm oc* $ps^{58k}$ ♀ × *cm oc* $ps^{58k}$ ♂ | 5981 | 22.3 | (21.2) |
| VI | *sb–it* | *sb it/+ +* ♀ × *sb it* ♂ | 792 | 27.7 | |
| | | *+ +/sb it* ♀ × *sb it* ♂ | 1959 | 29.9 | (29.3) |

* The numerator of a hybrid formula indicates the chromosome from maternal origin, and the denominator is of paternal origin.

*ge ro* as 24.4, 10.6 and 34.2 respectively, and the sum of 24.4 (*bwb–ge*) and 10.6 (*ge–ro*) agrees satisfactorily with the value for the distance between *bwb* and *ro*. The recombination value between *bwb* and *cp* is 34.2, but this figure may not be accurate because of rather poor penetrance of the *cp* gene.

Only two marker genes, *ocra* (ocra-eye) and *Lp* (Loop wing vein), were known in the 3rd linkage group. The recombination ratio is 39.9 percent between these two loci.

Attempts to synthesize a double recessive mutant stock with *ct* (cut wing) and *rb* (ruby eye) of the 4th linkage group have been carried out repeatedly for over two years but without success. The *ct* and *rb* genes appear to be located too close for crossing over to occur between them.

Four loci of the 5th linkage group, *cm* (carmine eye), *car* (carnation eye), *oc* (ocelliless) and $ps^{58k}$ (pigmented-sternites$^{58k}$), were studied. By a three point linkage test with triple recessive mutant *cm oc* $ps^{58k}$, the gene arrangement of *cm–oc–*$ps^{58k}$ was shown. The locus of *car* was demonstrated to be between *cm* and *oc*, from two independent tests with double recessive mutants *cm car* and *car oc*. Both *cm* and *car* are bright red eye color and their phenotypes overlap to some extent. However, the homozygote for both genes shows a pale pink eye color and is clearly distinguishable from *cm* or *car* individuals. The two genes are closely linked; 4.3 percent recombination was observed. Four different crosses to determine the recombination ratio between *cm* and *oc* gave similar results, and 43.7 percent is the value from the pooled data. A 48.4 percent recombination frequency is calculated for the distance between *cm* and $ps^{58k}$; thus crossing over is essentially free between them. Actually, the distance between *cm* and *oc* is at least 43.7 and the distance between *oc* and $ps^{58k}$ is 21.2; therefore, the sum of these two values is far more than 50. The two loci of *car* and *oc* are located far away from each other; 40.2 percent is the recombination ratio.

Two wing-vein recessive mutants, *sb* (subcostal-break) and *it* (interrupted), were the only marker genes found for the 6th linkage group; their recombination ratio is 29.3 percent.

## DISCUSSION

In the above experiments no indication of crossing over in male flies was found for the 2nd, 3rd and 5th linkage groups. MILANI and TRAVAGLINO (1957) and SULLIVAN (in press) observed exceptional occurrences of recombination among several loci of the 2nd linkage group, when heterozygous males were backcrossed to homozygous mutant females. The recombination ratios ranged from 0 to 3.46 percent depending upon the loci tested and also upon the strains used. However, since no observation on the genetic behavior of those recombinants in subsequent generations has been reported, nor has any cytological demonstration been offered by these investigators, one cannot be certain whether the "recombinants" were the results of crossing over in the males or of other causes. At any rate, there is no doubt that the house fly, *Musca domestica* L., belongs to the rare group of organisms in which no crossing over or only a very low frequency of crossing over is found in one of the sexes.

The linkage map of the house fly was therefore prepared on recombination values obtained by using heterozygous female flies (Figure 1). One of the terminal loci in a linkage group, or the locus of a marker found earlier than the other was chosen as the origin (0.0). *w* (white) is used as locus 0.0 for the map of the 2nd linkage group, *ocra* (ocra) for the 3rd, *ct* (cut) for the 4th, *cm* (carmine) for the 5th and *sb* (subcostal-break) for the 6th linkage group. Then, the map loci

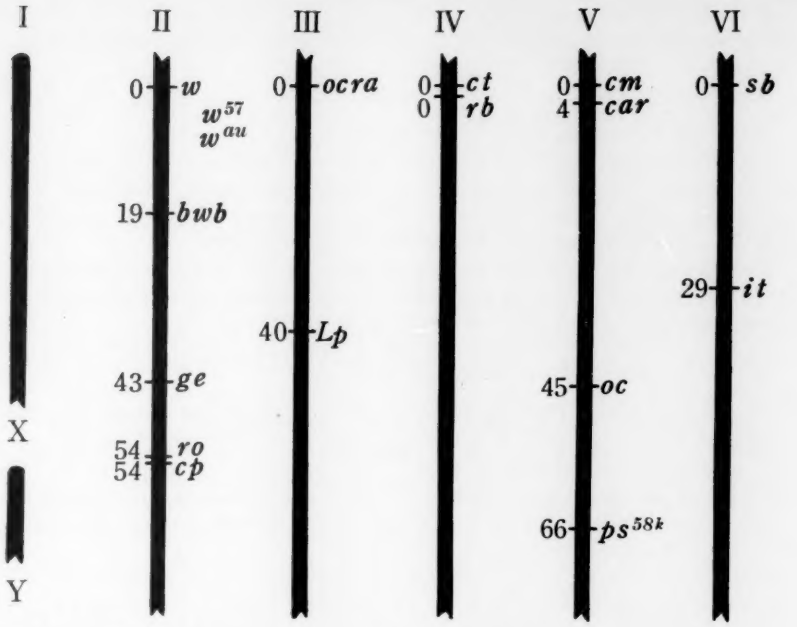


FIGURE 1.—The linkage map of the house fly, *Musca domestica* L.

were determined in the ordinary manner, by using the recombination values between adjacent genes. The linkage map presented in this paper is very simple with only a few marker genes being used. The genetic distances calculated in this paper should be reconsidered when more data on more marker genes become available. The author, however, hopes that this simple linkage map will be improved very soon, together with findings of more new mutants, and that it will encourage the future study of Musca genetics.

During the course of this study, males causing a sex-limited inheritance of the mutants on chromosome II were found; that is, when heterozygous males derived from a cross between a wild type male and a triple recessive mutant *bwb ge ro* female were backcrossed to homozygous mutant females, some pairs produced only mutant type females and wild type males rather than the usual segregation ratio. Similar phenomena of sex-limited inheritance of mutants of chromosome II of the house fly were found in American strains by MILANI and FRANCO (1959), by SULLIVAN (1958, 1961) and by HIROYOSHI (1960) and also in a strain in Japan by TSUKAMOTO *et al.* (1961). According to the genetical analysis, these segregational abnormalities seemed to be caused by translocations of a part of or the entire Y chromosome to one of the autosomes. Recently, KERR (1960) reported a case of sex-limited inheritance of DDT-resistance in an Australian strain of the house fly. Thus, a probable translocation of the Y chromosome to an autosome

is of world wide occurrence in the house fly, and the study on this subject seems important even in connection with the study of insecticide resistance of the insect.


## SUMMARY

A linkage map of the house fly, *Musca domestica* L., based on recombination values in the female, is presented in this paper. All five autosomal linkage groups are included. No marker gene has yet been found on the sex chromosome. Relative genetic distances among five loci of the 2nd linkage group, two each of the 3rd, 4th and 6th linkages and four of the 5th were studied.

Experiments attempting to detect crossing over in the male fly were performed with negative results. During these studies, males with a sex-limited inheritance of mutants on chromosome II were found.



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